

EFFECTS OF PLANT GROWTH REGULATORS AND THEIR INTERACTIONS
ON RESPIRATION, ETHYLENE EVOLUTION, COLOR DEVELOPMENT,
PEROXIDASE ACTIVITY, AND PEROXIDASE ISOENZYME PATTERN
OF DETACHED TOMATOES

By

SHUE-CHENG LIN

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1978

DEDICATED WITH LOVE

to my parents

Fu-chien Lin
and
Chin-tzu Cheng-Lin

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. D. D. Gull, Chairman of the supervisory committee, for his concern, unselfish efforts, and energies during the course of these studies and during the preparation of this manuscript.

Deep appreciation is extended to Dr. L. C. Hannah for his stimulating guidance and discussion during the course of this research. To Dr. B. D. Thompson, the initial chairman of my committee, for his encouragement during the course of this investigation. To Dr. H. B. Clark and Dr. R. C. Smith for their interest and kindly service on my committee.

I am very grateful to my sponsor, JCRR, for two and one half years fellowship and USAID for their arrangement and negotiation to study at the University of Florida.

Above all, the warmest expression goes to my wife, Lih-shoue Shih, for her untiring help and constant sacrifice; to my parents-in-law for their taking care of my two sons in Taiwan during the graduate years.

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS.....	iii
LIST OF TABLES.....	vii
LIST OF FIGURES.....	ix
ABSTRACT.....	xiii
INTRODUCTION.....	1
LITERATURE REVIEW.....	3
Some Factors Affecting Fruit Ripening.....	3
Fruit Maturity.....	3
The Climacteric Respiration.....	4
Plant Growth Regulators.....	7
Changes of Plant Hormone Level During Fruit Development.....	7
Exogenous Plant Growth Regulators vs. Fruit Ripening....	7
Auxin.....	7
Gibberellins.....	8
Cytokinin.....	9
Absciscic acid.....	10
Ethylene.....	11
Peroxidase and IAA Oxidase.....	12
Molecular Site of IAA Oxidase.....	12
Cellular Location of Peroxidase and Indoleacetic Acid Oxidase.....	13
Behavior of Peroxidase and IAA Oxidase During Ripening of Fruit.....	13
Effects of Hormones on Peroxidase and IAA Oxidase Activity and Their Isoenzymes.....	14
'Rin' Tomato— A Non-Ripening Mutant.....	15
MATERIALS AND METHODS.....	17
Experimental Design.....	17
Tagging and Pruning.....	17
Harvesting and Handling.....	17
Vacuum Infiltration of Fruit Treatment Solutions.....	18
Concentration of Treatment Solutions.....	18
Measurement and Determination.....	20
Color Grade.....	20
Carbon Dioxide.....	20
Ethylene.....	22

	Page
Enzyme Activity Analysis.....	22
Enzyme Preparation.....	22
Assay of Enzyme Activity.....	23
Gel Electrophoresis.....	24
Electrophoresis Preparation.....	24
Gel Staining.....	24
Peroxidase.....	24
IAA Oxidase.....	25
RESULTS.....	26
Respiration Rate, Ethylene Evolution, and Color Development..	26
Comparisons of Respiration Rate, Ethylene Evolution,	
and Color Development.....	26
Effect of Plant Growth Regulators and Protein Synthesis	
Inhibitors on Respiration Rate, Ethylene Evolution,	
and Color Development.....	26
IAA and its combinations.....	50
GA ₃ and its combinations.....	52
Kinetin and its combinations.....	53
ABA and its combinations.....	54
Ethrel and its combinations.....	54
Protein synthesis inhibitors.....	55
Protein Content and Enzyme Activity.....	55
Partial Purification of Enzyme Extracts.....	55
Enzyme Characteristics.....	57
Dilution and incubation test.....	57
Induction effect.....	57
Comparison of Protein Content and Peroxidase Activity	
by 'Floradel' and 'Rin' Tomato at Designated Stages	
of Color Development.....	57
Effect of Plant Growth Regulators on Protein and	
Peroxidase Specific Activity of 'Floradel' Tomato	
Fruit at Designated Stages of Color Development.....	62
Electrophoresis and Isoenzyme Pattern (Zymogram).....	72
Comparisons of 'Floradel' and 'Rin' Tomato at	
Designated Stages of Color Development.....	72
Effect of Plant Growth Regulators on the Isoenzyme	
Pattern of 'Floradel' Tomato at Designated Stages	
of Color Development.....	73
Mature-green (m-G) stage.....	73
Breaker stage.....	77
Turning stage.....	77
DISCUSSION.....	93
Methodology.....	93
Respiration, Ethylene Production, and Color Development.....	93
Protein Synthesis and Peroxidase Activity.....	98
Isoenzymes vs. Plant Growth Regulators and Protein	
Inhibitors.....	100

	Page
CONCLUSION AND SUMMARY.....	103
APPENDIX.....	106
BIBLIOGRAPHY.....	109
BIOGRAPHICAL SKETCH.....	123

LIST OF TABLES

Table		Page
1	Ripening and ethylene evolution of 'Rin' tomatoes at 21° C.....	29
2	Respiration rate at 21° C of 'Floradel' tomato fruits treated with plant growth regulators and protein-synthesis inhibitors.....	30
3	Ethylene evolution at 21° C of 'Floradel' tomato fruits treated with plant growth regulator and protein synthesis inhibitors.....	32
4	Color development of 'Floradel' tomato treated with plant growth regulator and protein-synthesis inhibitors at mature-green stage, then held at 21° C.....	34
5	Ethylene evolution at 21° C of 'Rin' tomato treated with plant growth regulators and protein-synthesis inhibitors.....	35
6	Respiration rate at 21° C of 'Rin' tomatoes treated with plant growth regulators and protein-synthesis inhibitors.....	51
7	Activities of peroxidase and IAA oxidase from tomato enzyme extracts partially purified by ammonium sulfate fractionation and dialysis in phosphate buffer.....	56
8	Effect of dilution on IAA oxidation by tomato enzyme extracts between 60-70% ammonium sulfate saturation and dialysis in phosphate buffer.....	58
9	Effect of incubation time on the oxidation of IAA by tomato enzyme extract between 60-70% ammonium sulfate saturation and dialysis in phosphate buffer.....	59
10	Comparisons of protein content and peroxidase activity of 'Floradel' and 'Rin' tomato at designated stages of color development.....	61

11	Protein content and peroxidase activity of 'Floradel' tomato treated with plant growth regulators and protein-synthesis inhibitors at mature-green stage, then held at 21° C.....	63
12	Protein content and peroxidase activity of 'Floradel' tomato at breaker stage with plant growth regulators and protein-synthesis inhibitors at mature-green stage, then held at 21° C...	64
13	Protein content and peroxidase activity of 'Floradel' tomato at turning stage when treated with plant growth regulators and actinomycin D at mature-green stage, then held at 21° C.....	65
14	Protein content and peroxidase activity of 'Floradel' tomato at pink stage, when treated with plant growth regulators and actinomycin D at mature-green stage, then held at 21° C.....	66

LIST OF FIGURES

Figure		Page
1	Serum bottle sleeve fitted over the stem scar of tomato and bonded to the fruit with paraffin wax for vacuum infiltration of treatment solutions.....	19
2	Vacuum infiltration of the treatment solution through the rubber serum bottle sleeve.....	19
3	Various ripening stages of 'Floradel' tomato fruits 1. Mature-green, 2. Breaker, 3. Turning, 4. Pink, 5. Red.....	21
4	Comparisons of respiration rate and ethylene evolution by 'Rin' and 'Floradel' tomato fruits at 21° C.....	27
5	Influence of IAA treatments on color development of 'Floradel' tomato fruits at 21° C.....	28
6	Influence of IAA treatments on respiration rate of 'Floradel' tomato fruits at 21° C.....	36
7	Influence of IAA treatments on ethylene evolution of 'Floradel' tomato fruits at 21° C.....	37
8	Influence of Gibberellin A ₃ treatments on respiration rate of 'Floradel' tomato fruits at 21° C.....	38
9	Influence of gibberellin A ₃ treatments on ethylene evolution of 'Floradel' tomato fruits at 21° C.....	39
10	Influence of gibberellin A ₃ treatments on color development of 'Floradel' tomato fruits at 21° C.....	40
11	Influence of Kinetin treatments on respiration rate of 'Floradel' tomato fruits at 21° C.....	41
12	Influence of Kinetin treatments on ethylene evolution of 'Floradel' tomato fruits at 21° C.....	42
13	Influence of Kinetin treatments on color development of 'Floradel' tomato fruits at 21° C.....	43
14	Influence of abscisic acid treatments on respiration rate of 'Floradel' tomato fruits at 21° C.....	44

Figure		Page
15	Influence of abscisic acid treatments on ethylene evolution of 'Floradel' tomato fruits at 21° C.....	45
16	Influence of abscisic acid treatments on color development of 'Floradel' tomato fruits at 21° C.....	46
17	Influence of protein synthesis inhibitors and Ethrel treatments on respiration rate of 'Floradel' tomato fruits at 21° C.....	47
18	Influence of protein synthesis inhibitors and Ethrel treatments on ethylene evolution of 'Floradel' tomato fruits at 21° C.....	48
19	Influence of protein synthesis inhibitors and Ethrel treatments on color development of 'Floradel' tomato fruits at 21° C.....	49
20	Induction effect after cessation of IAA oxidation by tomato enzyme extracts partially purified at 60-70% ammonium sulfate saturation and dialysis in phosphate buffer.	60
21	Influence of IAA treatments on peroxidase specific activity of 'Floradel' tomato at designated stages of color development.....	67
22	Influence of gibberellin A ₃ on peroxidase specific activity of 'Floradel' tomato at designated stages of color development.....	68
23	Influence of Kinetin on peroxidase specific activity of 'Floradel' tomato at designated stages of color development.....	69
24	Influence of abscisic acid treatments on peroxidase specific activity of 'Floradel' tomato at designated stages of color development.....	70
25	Influence of protein synthesis inhibitors and Ethrel treatments on peroxidase specific activity of 'Floradel' tomato at designated stages of color development.....	71
26	Isoenzyme pattern of 'Rin' tomato at mature-green, green-yellow, yellow-green, and yellow color stages compared with 'Floradel' tomato at breaker stage.....	74

Figure		Page
27	Mobility rate ($\pm R_f$) of anionic peroxidase isoenzymes of 'Rin' and 'Floradel' tomatoes after electrophoresis on polyacrylamide gels.....	74
28	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' and 'Rin' tomato at designated stages of color development.....	75
29	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with IAA treatments at mature-green stage.....	76
30	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with gibberellin A_3 treatments at mature-green stage.....	78
31	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with Kinetin treatments at mature-green stage.....	79
32	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with abscisic acid treatments at mature-green stage.....	80
33	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with protein synthesis inhibitors and Ethrel treatments at mature-green stage.....	81
34	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with IAA treatments at breaker stage.....	82
35	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with gibberellin A_3 treatments at breaker stage.....	83
36	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with Kinetin treatments at breaker stage.....	84
37	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with abscisic acid at breaker stage.....	85
38	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with protein synthesis inhibitors and Ethrel treatments at breaker stage.....	86

Figure		Page
39	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with IAA treatments at turning stage.....	87
40	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with gibberellin A ₃ at turning stage.....	88
41	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with Kinetin treatments at turning stage.....	89
42	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with abscisic acid treatments at turning stage.....	91
43	Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with actinomycin D and Ethrel treatments at turning stage.....	92
44	Blue dye penetration into fruit through vascular bundles by vacuum infiltration (A) and syringe injection (B)....	94

Abstract of Dissertation Presented to the Graduate Council
of the University of Florida in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

EFFECTS OF PLANT GROWTH REGULATORS AND THEIR INTERACTIONS
ON RESPIRATION, ETHYLENE EVOLUTION, COLOR DEVELOPMENT,
PEROXIDASE ACTIVITY, AND PEROXIDASE ISOENZYME PATTERN
OF DETACHED TOMATOES

By

Shue-cheng Lin

December 1978

Chairman: D. D. Gull

Major Department: Horticultural Science (Vegetable Crops)

The respiration, ethylene evolution, and color changes of tomatoes (cv. 'Floradel') treated with plant growth regulators appeared to follow a predetermined pattern. Gibberellin A_3 (GA_3), Kinetin, and Indoleacetic acid (IAA) in decreasing order of effectiveness inhibited ripening of tomato when treated alone or in combination, whereas abscisic acid (ABA) and Ethrel hastened ripening. Antagonistic effects on respiration and ethylene evolution resulted from the combination treatment of GA_3 or Kinetin plus ABA or Ethrel, whereas synergistic effects were found in the GA_3 +Kinetin treatment.

Peroxidase activity in control treatment increased about 3 times as fruit ripened from mature-green to the turning stage. Gibberellin A_3 and Kinetin decreased the enzyme activity, whereas IAA, ABA, and Ethrel increased activity in both mature-green and breaker stage. Increase or decrease of the enzyme activity was related to the onset of the climacteric and color development.

The isoenzyme pattern of peroxidase can be influenced by the plant growth regulators. An isoenzyme c which was affected by IAA in high

concentrations ($5 - 5 \times 10^{-2}$ mM) appeared when fruit attained the breaker stage. A rapidly migratory isoenzyme i and a slowly migratory isoenzyme b did not exist in 'rin' mutant when compared to 'Floradel' tomatoes. It is suggested that isoenzyme c is related to fruit ripening and isoenzyme i relate to the inhibition of ripening in 'Rin' tomatoes.

Indoleacetic acid oxidase and peroxidase were demonstrated to be the same enzyme by electrophoresis and enzyme activity levels in the course of ammonium sulfate fractionation.

'Rin' tomatoes treated with growth regulator showed no ethylene evolution or respiration stimulation except a temporary rise in respiration with the Ethrel treatment. Growth regulator did not affect the peroxidase activity, and this may be the reason why ethylene remained unchanged.

Protein inhibitors decreased protein content in both 'Floradel' and 'Rin' tomatoes. Cycloheximide inhibited the peroxidase activity but actinomycin D did not. The results suggested that m-RNA for peroxidase synthesis preexists in the cell.

A hypothesis is proposed that fruit ripening is caused by changes in hormone pool sizes inside the tissue. A ripening-induced signal is generated when the composition of each constituent attains a proper ratio which can be modulated by different environments such as temperature, light, fertilizer, age, management, and exogenous plant growth regulators. The ethylene-forming essential enzyme(s) is thought to be the best candidate to accept this signal to initiate fruit ripening.

INTRODUCTION

Ripening plays an important role in quality and subsequent storage life of fruits. Many fruits require ripening for optimum quality and usually senescence is initiated soon thereafter. Thus, to reveal the cause of induction, fruit ripening is of interest in fruit handling. It has been proposed that a "plant hormone pool" controls fruit ripening (41). Examples include delayed lycopene synthesis in tomato slices due to gibberellin (43) contracted by earlier synthesis due to abscisic acid (86); inhibition of chlorophyll degradation in orange peel by the application of gibberellins or cytokinins (44), and promotion by endogenous or exogenous ethylene (6). Similarly, auxin inhibits softening and chlorophyll degradation in 'Bartlett' pear and in banana peel slices (50, 167).

Protein synthesis is increased during fruit ripening. In banana, protein both increased in the rate of synthesis and the nature of the protein complement changed (18). The latter is considered of special significance with respect to ripening and conceivably could occur without a net gain in protein content. Thus, the role of plant growth regulators on fruit ripening may be as a direct effect on specific enzymes or to pass a signal to DNA causing the transcriptional and translational process inducing fruit ripening.

Horseradish peroxidase has been found essential in ethylene synthesis through a methionine-methional-ethylene pathway (175). Similar to the

horseradish peroxidase system, cell-free and dialyzed tomato enzyme extracts catalyzed formation of ethylene from α -keto- γ -methyltiobutyrate substrate (93).

Conversely, it is possible that peroxidase represents IAA oxidase and, thus, to postulate that the reduction of auxin in tissue is due to the activity of this enzyme. It has been confirmed that IAA oxidase activity is due to peroxidase (146). Plant hormones can also affect the peroxidase activity. Ethylene increases peroxidase activity in sweet potato root disk (56); gibberellin mediates IAA level in cucumber seedling by regulating its peroxidase activity (73). Kinetin had profound effects on IAA oxidase and peroxidase in tobacco callus tissue (100). In addition, abscisic acid was found to increase endogenous ethylene which might indirectly affect this enzyme in tomato fruit (86). Therefore, peroxidase would be the best candidate to study the relationship between plant growth-regulators and enzymes involved in fruit ripening.

The primary objective of this study was to establish the relationship between peroxidase and tomato fruit ripening when it is affected by plant growth regulators. Data are presented on ripening parameters of tomato, total peroxidase activity and changes in isoenzyme pattern (zymogramme) of peroxidase during fruit ripening.

LITERATURE REVIEW

The historical development of fruit ripening can be traced through a series of treatises on the various aspects of ripening including respiration (12), gas exchange (162), physiological role of ethylene (1, 23, 127), metabolism during storage (75), hormonal control of ripening (41), and senescence (136, 166).

Relevant reviews are readily available concerning fruit development and ripening as affected by growth regulators (33, 119), auxin (46, 61, 138), ethylene (1, 22, 108, 127), Kinetin (118, 148, 154), gibberellins (82), and abscisic acid (117).

Some Factors Affecting Fruit Ripening

Fruit Maturity

In non-climacteric fruits, no appreciable lag period between maturation and ripening can be distinguished; however, with climacteric type fruit a lag is evident. As fruits mature, there is an increase in sensitivity to ethylene (24). The change in threshold values for ethylene stimulation may be associated with a synthesis of specific enzymes required for ripening. Other factors associated with resistance to ripening have been suggested and the possibility exists that a ripening inhibitor is translocated into the fruit, or a substance capable of promoting ripening forms in the fruits but is maintained at a low concentration prior to maturity (26, 35). In banana tissue, a marked change in membrane

permeability is initiated about two days before the onset of the climacteric manifested first by a gradual increase and then by a rapid increase in intercellular space (135). A hypothesis has been presented that permeability changes are the cause of the climacteric (14). However, it has been found that leakage does not increase until well after the climacteric rise in banana tissue. Therefore, it may be concluded that permeability changes are not necessarily a prerequisite to ripening (17). Frenkel (48) postulated that auxin level declines and IAA oxidase activity increases during fruit maturation and ripening, lowering the threshold value for ethylene-stimulated ripening. This hypothesis was tested by applying an antiauxin, α -(p-chlorophenoxy) isobutyric acid (CIPA), to intact 'Bartlett' pears to inactivate endogenous auxins in fruit, thereby initiating an ethylene-independent ripening (53). Application of exogenous auxin could overcome the action of ethylene and inhibit ripening (50). A further suggestion was made that oxidative degradation may represent a mechanism for the inactivation of endogenous auxins in fruit and thereby the promotion of ripening, and the products resulting from the oxidative breakdown of auxins may function as regulatory factors in the enhancement of ripening (49).

The Climacteric Respiration

Since Kidd and West (88) demonstrated a climacteric rise in respiration in ripening fruit, and Biale (12) introduced the concept of climacteric fruits and non-climacteric fruits, much effort has been expended in seeking to define and explain the climacteric per se (153). Biale (13) concluded that the mechanism for energy generation is operational throughout the climacteric. Reid and Pratt (129) also reached a similar conclusion that the

respiratory climacteric is the respiratory summation of cellular energy requirement. However, Sacher (136) pointed out the absence of evidence that the energy requirements for ripening of non-climacteric fruits is less than that of climacteric fruits.

Considerable progress has been made in recent years in studying the cause of induction of climacteric respiration. Ethylene stimulation of color development in tomatoes is prevented by gibberellic acid, but the stimulation of the respiratory climacteric is not (43). McGlasson et al (115) studied the effect of a number of inhibitors on banana ripening and concluded that cycloheximide inhibited ripening but not the respiratory climacteric, indicating that the climacteric rise is not dependent upon, and integrated with, other aspects of ripening. On the other hand, compounds which inhibited the respiratory climacteric invariably inhibited ripening, indicating that some essential non-respiratory components of ripening are dependent on the respiration rise or on the factors which lead to it. Blanpied and Hansen (15) showed that pears did ripen at low oxygen concentration (2,5% O_2) without an increase in respiration. Wang and Hansen (171) suggested that the climacteric may have no unique function in ripening and that its biochemical significance and relationship to senescence is in need of reconsideration. Reid and Pratt (130), after reviewing recent work and their own study on the effect of ethylene on potato tuber respiration, concluded that the respiratory climacteric is not the primary event of ripening; instead, it is a parallel event, induced by endogenous ethylene.

A better understanding of climacteric respiration is studied in terms of respiratory chain in mitochondria. Van Herk (164) first

discovered the marked resistance of Sauromatum guttatum spadix respiration inhibition by cyanide and recorded the exceptionally high respiration rate of the tissue after the opening of the spathe. An active cyanide-resistant respiration is associated with mitochondrial preparation from the Arum spadix (81). Bendall and Bonner (11) developed a concept of non-phosphorylative, cyanide-insensitive respiratory pathway in plants which is mediated by an alternate oxidase in the mitochondria and interacts directly with the 'main' respiratory chain containing the cytochrome oxidase. Ubiquinone is the carrier common to both the cytochrome and alternate oxidase pathways linked to flavoprotein (158).

Similarities exist between the actions of ethylene and cyanide in initiating the climacteric and ripening of avocados (149) and the same situation exists in potato tubers (150). In both cases, glycolysis was enhanced and the gases produce seemingly identical physiological and biochemical changes. They concluded that ethylene enhances plant respiration, that the cyanide-insensitive, or alternate pathway, must be present and potentially operative, and that this cyanide-resistant electron transport is found in climacteric fruit (avocado, cherimoya), but not in non-climacteric tissue (green bean). They also indicated that the ATP increase during climacteric is due to the increased electron flow through site 1, together with the increase in substrate level phosphorylation, and is not due to the activation of pyruvate kinase and phosphofructokinase (151). Dizengremel and Lance (42) working with potato tuber indicated that an alternate, non-phosphorylative, cyanide-insensitive electron transfer pathway was brought into operation with aging and that only the cytoplasmic protein-synthesizing system has

an effect on the changes in mitochondrial properties during aging since cycloheximide prevented the development of the cyanide-resistant electron transport pathway as well as the enhancement of NADH oxidation.

Plant Growth Regulators

Changes of Plant Hormone Level During Fruit Development

To study the effect of an exogenous hormone treatment, it is important to establish whether it is endogenously present as well as the nature and persistence of effects on the tissue at the time of exogenous treatment (27).

Few papers have reported the changes of endogenous plant hormones in tomato fruits during development and maturation. Bioassay of cherry tomato fruits established that a sigmoidal relationship existed between fruit development and hormone levels. Cytokinin, IAA, and gibberellins attained their maximum content at 2, 3, and 4 weeks, respectively, after anthesis, while ABA content increased gradually during fruit growth (4). Although fruits exhibit the characteristic sigmoid growth, it is found that IAA, cytokinin, and gibberellins showed a more or less bimodal profile with the exception of endogenous ethylene which was high in the early stage of growth and gradually decreased until fruit maturation. The increase of GA levels that preceded auxin increase suggested that gibberellins enhance auxin synthesis or IAA oxidase activity. Cytokinin increased earlier than auxin or gibberellins (45).

Exogenous Plant Growth Regulators vs. Fruit Ripening

Auxin. Several laboratories have shown that applied auxins accelerate rather than retard the onset of fruit ripening in pear, banana, fig and tomato (39, 74, 77, 110). These results are contrary to more

recent information and may be explained on the basis of uneven penetration of auxin where dipping was employed. Vendrell (168) dipped intact banana into radioactive 2,4-D solution and found that ethylene production was stimulated and pulp ripening proceeded rapidly, but peel ripening was delayed. These results were ascribed to auxin penetrating the peel, but not into the pulp tissue. The dual effect of auxin could also lead to this ambiguous result. Tingwa and Young (160) stated that a high concentration (100 & 10000 μM) of IAA stimulated respiration and induced preclimacteric ethylene production resulting in accelerated ripening of avocado fruit; low concentration (10 μM) delayed ripening.

More recently, auxin was recognized as a senescence retardant because of its ability to desensitize fruit to exogenous or endogenous ethylene in banana, pear, avocado, and grape (50, 72, 160, 167) and this is hypothesized by stimulating the action of "juvenility factors" in the fruit tissue (51). It is more generally agreed that exogenous auxin has a senescence-retarding action at the time when endogenous auxin declines in the fruit.

Gibberellins. It has become evident that gibberellic acid delays fruit maturation and ripening. Postharvest application of gibberellin to tomato fruits or tissue slices delayed ripening and also decreased cellulase and polygalacturonase (9, 43). Gibberellins also delay ripening in other fruits such as banana, guava, kaki fruit, citrus, apple, and retard senescence of Romaine lettuce (5, 63, 89, 124, 134, 159, 163).

The effects of gibberellin in the regulation of ripening can be in blocking the pigment changes during ripening. Gibberellins might serve as inducers of chloroplast formation and not just protection of

the chloroplast from degradation (28). Gibberellin could defer ripening, i.e., the development of red color without any apparent alteration of the climacteric (43). Similar reactions were found in banana (169). On the other hand, Scott and Leopold (139) suggested that gibberellins act in fruits in indirect opposition to ethylene that has been shown in several growth and enzyme systems.

Cytokinin. Kinetins, Benzyladenine (BA), and cytokinins were shown to delay chlorophyll degradation and senescence of leafy vegetables by retardation of yellowing and by maintaining high protein levels in the applied tissue (5, 103, 132). Recently, cytokinin was found to stimulate protein synthesis in in vitro system prepared from tobacco and corn shoots (90).

Postharvest treatment of freshly harvested sweet cherries with BA resulted in more attractive and greener fruit pedicels and less loss of fresh weight of the fruit (161). Abdel-Gawad and Romani (2) observed that BA retarded the rate of ripening of apricots when applied as a postharvest treatment. Kinetin and BA were reported to delay tomato ripening as expressed by color development, decreasing respiration and inhibition of lycopene synthesis (3, 87).

More recent work has shown that cytokinins have a depressing action on respiration. Inhibition of respiration and senescence by BA has been reported in freshly harvested broccoli, Jonathan apples, chrysanthemum, and carnation flower stalks (40, 41, 106). Furthermore, Kinetin was found to inhibit oxygen uptake in pea stem sections and in excised wheat leaves (145). Ballantyne (10) questioned whether the cytokinin-induced decrease in respiration is of prime importance in delaying tissue senescence. However, Kinetin with CA^{++} were found

to stimulate ethylene production in hypocotyl of mung bean (97). The requirement for Ca^{++} in this system was specific (99). It is suggested that the synergism between Kinetin and Ca^{++} on ethylene production is due to the enhanced intake of Ca^{++} by Kinetin into a specific site for ethylene production (98).

Absciscic acid. Recent research indicated that senescence was correlated with an increase in content of ABA in both climacteric and non-climacteric fruits, and in leaves (29, 45, 62). Application of abscisin accelerates senescence in detached fruits and leaves. Cooper and Henry (31) reported that ABA treatment of oranges resulted in accelerated breakdown of chlorophyll and increased synthesis of carotenoids. Ripening of banana slices was also accelerated by abscisic acid (168); ABA hastened senescence of carnation flowers, preceded by accelerated ethylene production (111).

Khudairi (86, 87) hypothesized that tomatoes can be ripened by phytochrome-mediated ABA and ethylene, and the site of action of ABA is not the same as for ethylene. Ethylene enhances chlorophyll degradation and the formation of phytol which is inhibited by gibberellin; ABA affects the synthesis of lycopene from neurosporene which is inhibited by temperatures of 30° C or higher. Application of ABA into tomato fruits by vacuum infiltration enhanced ripening of tomato as effectively as ethylene and Ethrel (83).

Fruit has the capacity to rapidly synthesize ABA or convert an inactive ABA-glucoside to ABA which then enhances ethylene production (32). On the other hand, ethylene-increased ABA content was produced in citrus flavedo (21). The site of synthesis of ABA was inside any type of plastids, not just in chloroplast. Absciscic-acid-accelerated

senescence may possibly promote ribonuclease degradation and may not necessarily inhibit ribonucleic acid (RNA) polymerase (101, 102).

Auxin, cytokinin, and gibberellins invariably have an effect opposite to those of ABA in various enzyme systems (34, 37, 38, 165). It is, therefore, suggested that ABA may serve as a negative allosteric effector on biologically active protein, where the positive effector may be gibberellins, cytokinin or auxin (137). Milborrow (117) suggested that RNA polymerase is an ABA-effected allosteric protein since a series of experiments indicated that ABA prevented incorporation of labelled uridine and thymidine into nucleic acids while no inhibition of amino acid incorporation into protein was experienced. This model outlines how in vivo growth may be the function of equilibrium attained between stimulatory and inhibitory factors,

Ethylene. The concept that ethylene is a ripening hormone was first proposed by Kidd and West in 1933 and was renewed by Burg (22). Proof that ethylene is essential for fruit ripening was shown by Burg and Burg in 1966 (25). In studies at reduced pressures, they showed conclusively that without ethylene, there was no ripening.

Studies have shown that as fruits mature there is an increase in sensitivity to ethylene (33). Also there is a decline in the concentration of exogenous ethylene required to initiate ripening as maturation progresses in pear and apple (76, 172). This phenomenon is ascribed to the existence of a ripening resistant substance in fruit. Tingwa and Young (160) indicated that the peduncle and stem may supply a ripening-inhibitor to the fruit or may act as a sink for a ripening hormone produced in the fruit. A ripening-inhibitor has recently been suggested to be one or more of auxin, cytokinin, and gibberellin varying with different kinds of fruit (85, 136, 167, 168, 169, 170).

The involvement of ethylene in ripening suggests that ethylene initiates ripening which requires oxygen, but is inhibited by carbon dioxide (26). Carbon dioxide accumulating to 4% in a ripening room competitively inhibits the action of ethylene in initiating the ripening of tomato (69). The latent potential for ripening is expressed when ethylene supposedly activates the synthesis of messenger RNA required for the synthesis of specific enzymes or sets of enzymes involved in the ripening complex, since protein synthesis was shown to be essential for normal ripening of apple (55).

Doubt has now been raised about the primary role of ethylene in fruit ripening. McGlasson et al. (114) reported propylene, like ethylene, stimulated the respiratory climacteric in tomato fruits, but ethylene production did not increase until ripening commenced. Similarly, the effects of reduced atmospheric pressure on the time of ripening of tomato fruits were not related to the amounts of ethylene present (157, 159). In both papers it was suggested that ethylene production is an integral part of ripening, but it does not act as a trigger (30).

Peroxidase and IAA Oxidase

Molecular Site of IAA Oxidase

It has been known since early 1950 that IAA can be oxidized by plant peroxidase (58). It was found that a peak of IAA oxidase did not show peroxidase activity in tobacco root (142); however, Shinshi and Noguchi (146) concluded that IAA oxidase activity was entirely due to peroxidase. It appears that both peroxidase and IAA oxidase can exist in multiple molecular forms and the isoenzymes of peroxidase

exist at various levels of IAA oxidase activity (138). When the heme prosthetic group of horseradish peroxidase (HRP) was removed by treatment with acidified acetone, the apoenzyme still had IAA oxidase activity towards peroxidase substrates such as guaiacol (147), but Ku, Yong and Pratt have found that if the prosthetic group of the HRP was removed, the apoenzyme had almost no IAA oxidase activity (94).

Cellular Location of Peroxidase and Indoleacetic Acid Oxidase

Most peroxidase of the cell is soluble, but peroxidase may also be associated with the cell wall and with various subcellular organelles. Cytochemical studies have shown that peroxidase activity may be associated with many of the membranous components of the cell, such as the plasmalemma and the tonoplast, and that peroxisomes give a particularly strong peroxidase reaction (122). Plesnicar (125) found that 90% of the peroxidase activity of mung bean seedlings was water soluble, 6% was associated with microsomes and about 0.5% was in the mitochondrial fraction. Activity was not associated with the mitochondria per se, but with a denser, lysosomal-like particle which contaminated the fraction. While Ku et al. (94) claimed there is no peroxidase activity in mitochondria, others have found IAA oxidase and peroxidase in all subcellular fraction of tobacco callus cells (100).

Behavior of Peroxidase and IAA Oxidase During Ripening of Fruit

Recent investigations demonstrated that peroxidase activity increased with ripening of mango, grape, apple, and pear (80, 96, 109, 128). Soluble peroxidase in ripening banana fruit remained relatively constant when extracted from pulp; however, particulate peroxidase levels increased 3-fold with initiation of the respiratory climacteric and gradually declined with the onset of senescence (70, 71). In

climacteric fruit (tomato, pear), peroxidase and IAA oxidase increases activity during fruit ripening, but in conventional non-climacteric fruit (blueberry), IAA oxidase activity increases whereas peroxidase activity decreases ripening (48). Blueberry has recently been found as a climacteric fruit (173).

As tomato fruits ripened, the peroxidase activity increased 3 to 4 fold (95); this is also shown in apples (123). The peroxidase activity curve for apple fruits corresponds to the respiration activity. As a function of storage time, peroxidase of 'Golden Delicious' apple gives two peaks, the first corresponding to climacteric and the second to the start of senescence (66).

Effects of Hormones on Peroxidase and IAA Oxidase Activity and Their Isoenzymes

There is a multitude of peroxidase isoenzymes, and their number and relative concentration varies between different tissues and with the stage of plant development. Because peroxidase isoenzymes are responsive to hormonal levels, this enzyme system has been used as a model to study hormonal control of growth processes in plant (60). Plant growth hormones have never been shown to have a direct effect on peroxidase or IAA oxidase activity in vitro, but considerable effects on the levels of these enzymes in vivo have been demonstrated in a wide variety of tissues (138).

Little is known about the effect of plant hormones on the activity and isoenzymes of peroxidase and IAA oxidase. Matto and Modi (109) indicated that ethylene increased about three times as much as peroxidase activity when mango fruit attained the climacteric stage. However, ethylene did not increase the peroxidase activity of ten cultivars of sweet potato root disks (56). The increase was inhibited by actinomycin D and cycloheximide, suggesting de novo protein synthesis.

'Rin' Tomato--A Non-Ripening Mutant

'Rin' tomato was first described by Robinson and Tomes (133) as a spontaneous mutation in a breeding line. Fruits of the 'Rin' mutant remain green while normal fruits ripen and turn red. The mutant eventually turns a yellow color with little or no lycopene development. Genetic analysis showed that 'Rin' was conditioned by a recessive gene.

The physiology and biochemistry of this mutant have been extensively investigated to determine the fruit ripening mechanism. Herner and Sink (78) reported little or no change in ethylene or CO_2 production in 'Rin' tomato mutant fruit over a period of 120 days. Fruits from F_1 plants resulting from reciprocal crosses between 'Rin' and normal plants appeared to ripen normally, but evolved only one-third to one-half as much ethylene at the peak of production compared to normal fruits. Exogenous ethylene or propylene did not stimulate ethylene production by 'Rin' fruits but did stimulate CO_2 production. The CO_2 stimulation persisted only in the presence of the exogenous olefins. Stimulation of CO_2 production could be repeated several times in the same fruit. Wounding stress stimulated both ethylene and CO_2 production in 'Rin' fruit.

Mizrahi et al. (121) using stock-scion interactions of normal and 'Rin' mutant to study the influence of roots and vegetative tissue concluded that fruit did not ripen due to a non-translocatable ripening inhibitor. They also showed that endogenous levels of ABA and cytokinin did not account for the lack of ripening in 'Rin' mutant, since neither alter its respiration and ethylene production (120).

McGlasson found there was an apparent capacity for increased production of ethylene in aging leaf segments and in response to wounding in pericarp disks on 'Rin' but not in intact fruit during natural aging (116). This explained that a possible divergence of mechanism controlling ethylene synthesis in different tissues (176).

The first significant increase in tissue permeability of normal 'Rutgers' tomatoes had already begun with initiation of fruit ripening, but the 'Rin' did not show any increase in tissue permeability during fruit development or maturation (126).

Free methionine levels in mature-green fruits of both 'Rin' and normal tomatoes are similar. Therefore, lack of ripening of 'Rin' fruit is not due to low methionine levels (64).

High oxygen tensions can initiate the synthesis of lycopene and flesh softening in the 'Rin' tomato mutant. Therefore, it was suggested that depletion of auxin in ripening fruit is dependent on oxidative metabolism (49).

MATERIALS AND METHODS

Experimental Design

Experiments were initiated in the spring of 1976. Seed of tomato Lycopersicon esculentum, (Mill) cv. 'Floradel' and 'Rin' were sown in peat pots. Four-week old seedlings were transplanted into the field at the University of Florida Horticultural Unit at Gainesville. About 650 plants were planted in four rows with spacing 0.45 x 1 meter. Irrigation, fertilization, and plant protection sprays were applied according to established recommendations.

Tagging and Pruning

The plants, except 'Rin,' were pruned to maintain one or two central stems per plant and were topped at the fourth fruit-truss. The pruned plants were staked to facilitate fruit position identification, tagging, and observation. At each cluster generally two, but not more than three flowers were allowed to set. At anthesis the flowers were tagged and remaining flowers were removed to increase fruitset. Tagged flowers generally occupied the No. 2 and 3 flower positions.

Harvesting and Handling

Mature-green fruits were harvested three times according to the sequence of flower cluster on June 22, July 8, and 15 with the fruit age of 35, 30, and 30 days, respectively. Sampling was completed within 12 hours after harvest and only fruits that were blemish-free and uniform were selected for treatment. Fruits showing a color break

before treatment were discarded. Fruits were dipped in sodium hypochlorite at 500 ppm Cl^- for 4 min, rinsed with distilled water, and dried overnight at 21°C (70°F).

Vacuum Infiltration of Fruit Treatment Solutions

Sepals and stems were removed from fruit and a small rubber cup (serum bottle sleeve) was fitted over the stem scar and bonded to the fruit with paraffin wax (Figure 1). A volume of treatment solution, 1 ml per 100 g of fruit, was placed in the rubber cup and the tomato was placed under partial vacuum at 100 mm Hg. Upon release of vacuum the solution inside the rubber cup was gradually introduced into the fruit. About 20 min. were required for vacuum infiltration (Figure 2).

Concentration of Treatment Solutions

The following plant growth regulators and protein synthesis inhibitors were used in the experiment. Three replications were employed.

<u>Solution</u>	<u>Concentrations</u>
Indoleacetic acid (IAA)	5 mM $5 \times 10^{-2} \text{ mM}$ $5 \times 10^{-4} \text{ mM}$
Gibberellin A_3 (GA_3)	3 mM -- $3 \times 10^{-4} \text{ mM}$
Abscissic acid ³ (ABA)	2 mM -- $2 \times 10^{-4} \text{ mM}$
Kinetin (KN)	5 mM -- $5 \times 10^{-4} \text{ mM}$
Ethrel: 2-chloroethyl-phosphonic acid	500 ppm
Cycloheximide (C-H)	2.5 mg/l
Actinomycin D (Act.-D)	1 mg/l
IAA + GA_3	$5 \times 10^{-4} \text{ mM}$: $3 \times 10^{-4} \text{ mM}$
IAA + Kinetin	$5 \times 10^{-4} \text{ mM}$: $5 \times 10^{-4} \text{ mM}$
IAA + ABA	$5 \times 10^{-4} \text{ mM}$: $2 \times 10^{-4} \text{ mM}$
IAA + Ethrel	$5 \times 10^{-4} \text{ mM}$: 500 ppm
GA_3 + Kinetin	$3 \times 10^{-4} \text{ mM}$: $5 \times 10^{-4} \text{ mM}$
GA_3 + ABA	$3 \times 10^{-4} \text{ mM}$: $2 \times 10^{-4} \text{ mM}$
GA_3 + Ethrel	$3 \times 10^{-4} \text{ mM}$: 500 ppm
Kinetin + ABA	$5 \times 10^{-4} \text{ mM}$: $2 \times 10^{-4} \text{ mM}$
Kinetin + Ethrel	$5 \times 10^{-4} \text{ mM}$: 500 ppm
ABA + Ethrel	$2 \times 10^{-4} \text{ mM}$: 500 ppm
Distilled water as control and carrier	



Figure 1. Serum bottle sleeve fitted over the stem scar of tomato and bonded to the fruit with paraffin wax for vacuum infiltration of treatment solutions.



Figure 2. Vacuum infiltration of the treatment solution through the rubber serum bottle sleeve.

Measurement and Determination

Color Grade

Each tomato was scored for color at 2-day intervals. Color was based on an index from 0 (mature-green) to 5 (soft ripe) Figure 3. Each sample contained 20 tomatoes. Total sample score was calculated as product of color index x number of fruit.

<u>Color index</u>	<u>Ripening stage</u>
0	Mature-green: entirely green but mature (M-G).
1	Breaker (B) : first appearance of coloring at blossom end.
2	Turning (T) : approximately equal amount of green and pink.
3	Pink (P) : light pink.
4	Red (R) : light red color
5	Soft-ripe (S-R) : dark perceptable red color and softening

'Rin' tomato color was classified into four grades as 'green', 'green yellow', 'yellow green', and 'yellow'.

Carbon Dioxide

Sample size consisted of ten fruits held in a wide-mouth gallon glass jar maintained at 21° C (70° F). Atmospheric air, saturated with moisture, was continuously circulated through the closed system at a flow rate of 100 ml/min. The air stream was sufficient to allow normal respiration. Evolved CO₂ from the respiring tomatoes was measured with a Beckman Model 215 A infrared CO₂ analyzer and results recorded using a 24-point Westronics recorder.



Figure 3. Various ripening stages of 'Floradel' tomato fruits
1. Mature-green, 2. Breaker, 3. Turning, 4. Pink,
5. Red

Ethylene

Ethylene was measured by using a Hewlett Packard 5710 A gas chromatograph with a flame ionization detector. The column was 6' x 1/8" O.D., aluminum tubing packed with activated alumina. The flow rate of hydrogen and carrier gas (purified nitrogen) at the flame tip was 60 ml/min. Compressed air was supplied at a flow rate of 300 ml/min. at the flame tip. The oven temperature was maintained at 100° C, and the injector temperature was 150° C. Ten parts per million ethylene standard was used as the reference gas for calibration at time of each measurement. Sensitivity of ethylene detection was 5 ppb. A 1.0 ml gas sample was withdrawn into a gas tight plastic syringe from the effluent line of each fruit jar. Ethylene production was determined once every morning.

Enzyme Activity Analysis

Enzyme Preparation

After the fruit had been analyzed for color, a representative sample of 2-3 fruits were cut into sections, and the seeds and highly acid locular contents were removed. One hundred grams of fruit wall tissue were then put in polyethylene bags and stored at -29 to -32° C for not longer than 2 months. For enzyme analysis, the frozen fruit was allowed to thaw overnight at 5° C. All subsequent extractions were done in the cold room (5° C). The thawed slices from each sample were blended in a Waring blender with 25 ml of 0.1 M, pH 6.0 potassium phosphate buffer containing 2 mM EDTA and was mixed with 0.5% insolubilized polyvinylpyrrolidone (PVP) (92, 104). The slurry was pressed through 4-layer cheesecloth, and centrifuged at 30,000 x g for 20 min. The

supernatant was partially purified with ammonium sulfate. Precipitated protein was collected by centrifugation when the fraction was between 60-80% saturation, redissolved in 10 ml of potassium phosphate buffer 0.1 M, pH 6.0, and dialyzed against 50 ml of 0.1M, pH 6.0 potassium phosphate buffer for 12 hrs. exchanging the buffer after 3 and 10 hrs. This clear dialyzed enzyme solution was stored at 0° C and used to determine peroxidase and IAA oxidase activity and for disc gel electrophoresis. Protein content was measured by the Lowry et al. method (105).

Assay of Enzyme Activity

Peroxidase activity was measured by following the change in absorbance at 460 nm due to the oxidation of o-dianisidine in the presence of H_2O_2 (8). One unit of peroxidase activity is that amount of enzyme decomposing 1 μ mole of hydrogen peroxide per min at 25° C, assuming that the molar absorbance of oxidized o-dianisidine is equal to $1.13 \times 10^4 \text{ cm}^{-1}$ at 460 nm. The reaction mixture for assay of peroxidase activity consisted of 0.1 ml of diluted enzyme solution, 0.1 μ mole of o-dianisidine, 3 μ moles of H_2O_2 and 30 μ moles, pH 6.0 of potassium phosphate buffer in a total volume of 3 ml. Incubation was at 25° C and specificity is expressed as

$$\text{Units/mg} = \frac{A_{460/\text{min}}}{1.13 \times \text{mg protein/ml reaction mixture}}$$

For IAA oxidase activity determination, the standard reaction mixture contained 0.04 M potassium phosphate buffer at pH 5.5, 0.2 mM IAA, 0.1 mM 2,4-dichlorophenol, 0.1 mM $MnCl_2$, and 0.1 ml of diluted enzyme solution in a final volume of 1.0 ml. The reaction was carried out for 30 min in a shaking waterbath at 35° C. Two ml of modified

Salkowski reagent (65) was added and the absorbance was read at 530 nm after 30 min. Quantitative measurements of IAA in solution and rate of consumption were computed from a standard IAA curve. One enzyme unit was defined as 1 μ g IAA destroyed in 30 min at 35° C (146).

Gel Electrophoresis

Electrophoresis Preparation

An aliquot (0.2 ml) of the enzyme extract, prepared as described above, in 2% sucrose was applied to the top well of vertical gel just prior to electrophoresis. The amount of protein applied per gel was maintained at 15-30 μ g (for peroxidase) and 60 μ g (for IAA oxidase). Separation of the acidic proteins was carried out according to the discontinuous gel electrophoretic procedure of Davis (36) using a Buchler Polyanalyst Disc Electrophoresis Apparatus and Beckman Spino Duostat Power Supply. The gel columns (5 mm I.D., 65 mm long) were composed of 2 sections, the upper 5 mm of a 5.0% spacer gel and lower 60 mm of a 7.0% (W/V) running gel. The gel was currented for 2 hrs.

Gel Staining

Peroxidase. An aliquot of 1% o-dianisidine in absolute methanol was added to 0.2 M, pH 5.0 sodium acetate to give a final substrate concentration of 1 mM for the incubating solution. After an hour, the gels were taken from the incubating solution and rinsed in distilled water. They were then incubated in 0.2 M, pH 5.0 sodium acetate containing fresh 1.3 mM H_2O_2 for 90 min. After removal from this solution and rinsing with distilled water they were placed in 0.2 M, pH 5.0 sodium acetate. The isoenzymes of peroxidase appear as brown bands in the gels (141). Width of bands were recorded and their color intensity

determined by comparing with Munsell color system at 7.5YR 3/6, 7.5YR 4/8, 7.5YR 5/10, and 7.5YR 6/8 charts (7).

IAA Oxidase. The staining procedure was slightly modified from Gove and Hoyle method (67) by increasing the incubation time to 2 hours. Gels were incubated in a reaction mixture containing 4.6 ml of 2 M acetic acid, 0.4 ml of 2 M sodium acetate, 2 ml of H_2O , 2 ml of 4.5 mM p-coumaric acid, 2 ml of 6 mM H_2O_2 , and 2 ml of 1.5 mM IAA. After 2 hr at 35° C with periodic swirling, the gels were placed in 0.5% (W/V) p-N, N-dimethylaminocinnamaldehyde (DMACA) in 1 N HCl. The isozymes of IAA oxidase appear as red-violet bands in the gels. The bands were recorded by visual intensity.

RESULTS

Respiration Rate, Ethylene Evolution, and Color Development

Comparisons of Respiration Rate, Ethylene Evolution, and Color Development

Mature-green 'Floradel' fruits initiated the climacteric rise about 2 days after being treated with distilled water and attained the climacteric peak on the 6th day, whereas 'Rin' fruits decreased in respiration rate for 4 days, then stabilized thereafter for the duration of the experiment. Ethylene evolution was directly related to respiration changes in 'Floradel' fruit; however, 'Rin' tomatoes evolved a very low (almost zero) amount of ethylene during the experiment (Fig. 4). Color of 'Floradel' fruits remained green for 2 days, then changed to an advanced breaker stage after 4 days. The turning stage was attained after 6 days (Fig. 5). 'Rin' tomatoes stayed at the green stage for about 15 days (Table 1).

Effect of Plant Growth Regulators and Protein Synthesis Inhibitors on Respiration Rate, Ethylene Evolution, and Color Development

Data of growth regulator and protein synthesis inhibitor effects upon respiration rate, ethylene evolution, and color development of 'Floradel' and 'Rin' tomatoes are shown in Tables 2, 3, 4, and 5. Graphic presentation of those effects on 'Floradel' tomatoes are shown in Fig. 5-19. Plant growth regulators and protein synthesis inhibitors had no effect on ethylene evolution (Table 5), color development (data not shown) and respiration of 'Rin' tomato (Table 6) except that Ethrel

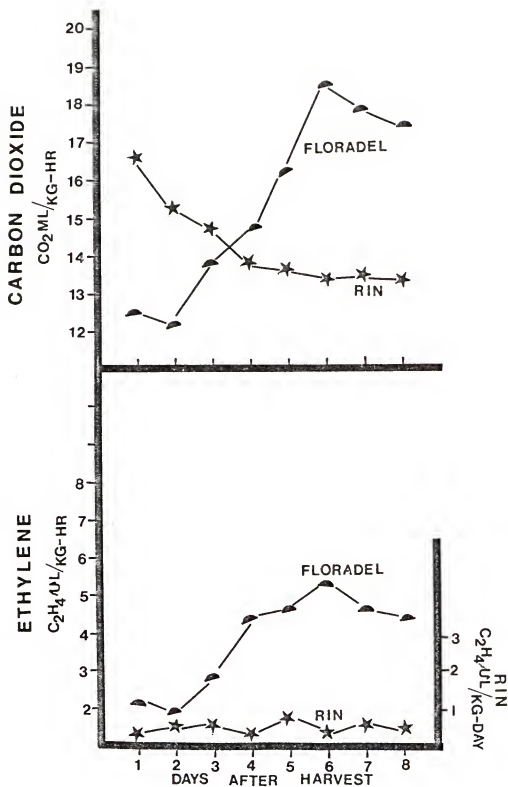


Figure 4. Comparisons of respiration rate and ethylene evolution by 'Rin' and 'Floradel' tomato fruits at 21° C.

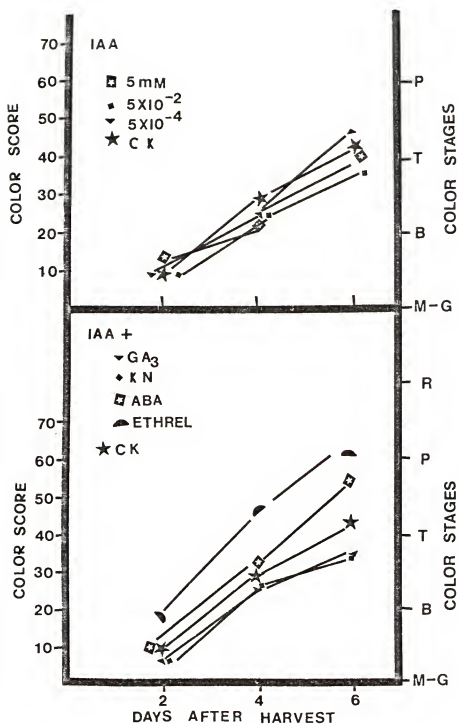


Figure 5. Influence of IAA treatments on color development of 'Floradel' tomato fruits at 21° C.

Table 1. Ripening and ethylene evolution of 'Rin' tomatoes at 21° C

Color Stage	Days from Harvest	Ethylene Evolution
		$\mu\text{l/kg/day}$
Green	15 ± 2^1	0.43 ± 0.08^1
Green-yellow	30 ± 8	0.51 ± 0.13
Yellow-green	61 ± 12	0.48 ± 0.10
Yellow	99 ± 20	0.58 ± 0.17

¹ Average of three replications with 5 fruits per sample, and standard deviation.

Table 2. Respiration rate at 21° C of 'Floradel' tomato fruits treated with plant growth regulators and protein-synthesis inhibitors.

Treatments	Days after harvest							
	1	2	3	4	5	6	7	8
	CO ₂ ml/kg/hr							
1. IAA 5 mM	15.32 ¹	14.83	14.43	14.26	15.80	17.87	20.02	18.57
2. IAA 5x10 ⁻² mM	14.29	14.23	14.61	15.67	16.61	17.32	18.66	18.00
3. IAA 5x10 ⁻⁴ mM	13.70	13.54	15.04	16.34	16.82	17.42	19.14	18.80
4. GA ₃ 3 mM	13.80	13.43	12.73	12.80	13.03	13.33	14.05	15.33
5. GA ₃ 3x10 ⁻⁴ mM	13.42	12.84	13.10	13.32	13.72	15.60	17.20	18.70
6. Kinetin 5 mM	12.63	12.54	12.44	14.00	15.71	17.10	18.41	19.20
7. Kinetin 5x10 ⁻⁴ mM	13.21	13.10	13.11	14.60	15.00	16.20	17.79	18.03
8. ABA 2 mM	14.09	14.33	15.55	20.23	18.60	17.57	17.40	17.10
9. ABA 2x10 ⁻⁴ mM	13.74	13.54	14.23	15.60	19.40	18.30	17.89	17.80
10. IAA + GA ₃	13.80	13.30	13.03	13.78	15.20	16.00	17.40	17.00
11. IAA + Kinetin	13.30	12.90	12.70	13.50	15.78	17.70	19.50	18.00
12. IAA + ABA	14.40	13.60	14.40	15.70	16.81	18.40	17.93	17.67
13. IAA + Ethrel	16.80	22.57	20.73	17.77	17.93	16.63	15.09	14.60
14. GA ₃ + Kinetin	13.15	12.40	11.91	12.00	12.70	14.00	16.17	16.00
15. GA ₃ + ABA	15.18	14.21	14.50	15.61	17.00	18.80	17.00	16.30
16. GA ₃ + Ethrel	16.39	20.02	19.25	18.00	16.58	15.77	15.19	14.81
17. Kinetin + ABA	11.80	10.80	11.50	12.41	15.40	17.50	16.54	16.30
18. Kinetin + Ethrel	15.00	16.68	18.63	20.97	19.46	18.33	17.01	16.60
19. ABA + Ethrel	18.10	22.90	20.33	19.54	17.76	16.37	15.43	15.00
20. Ethrel 500 ppm	17.90	23.20	21.64	19.33	17.94	17.33	16.20	15.03
21. Act-D 2.5 mg/l	13.40	7.80	5.60	4.90	10.96	16.70	14.70	15.00
22. C-H 1 mg/l	14.78	16.20	30.60	30.79	29.01	19.96	16.50	10.00
23. Control (water)	12.40	12.00	13.70	14.60	16.10	18.41	17.80	17.40
L S D	1.24	1.89	2.49	2.76	1.57	1.62	1.99	3.11

Table 2. continued (Waller-Duncan K ratio t-test).

Treatments	Days after harvest							
	1	2	3	4	5	6	7	8
1. IAA 5 mM	DE ²	CDE	DE	GHI	EFGH	BCDE	A	AB
2. IAA 5×10^{-2} mM	EFGHI	EFG	DE	FGH	DEFG	BCDEFGH	ABCD	ABC
3. IAA 5×10^{-4} mM	GHIJ	EFGH	DE	EFG	DEF	BCDEFG	ABC	AB
4. GA ₃ 3 mM	FAGIJ	EFGH	EFGH	IJ	J	K	J	CDE
5. GA ₃ 3×10^{-4} mM	HIJK	FAGI	EDFGH	HIJ	IJ	IJ	CDEFG	AB
6. Kinetin 5 mM	JKL	FAGI	FGH	GHIJ	EFGH	CDEFGHI	ABCDE	A
7. Kinetin 5×10^{-4} mM	HIJK	EFGH	DEFGH	GHI	HI	FAGI	BCDEF	ABC
8. ABA 2 mM	EFGHI	DEF	D	BC	BC	BCDEFG	CDEFG	ABCDE
9. ABA 2×10^{-4} mM	GHIJ	EFGH	DEFG	FGH	B	BCD	BCDEF	ABCD
10. IAA + GA ₃	FAGIJ	EFGH	EFGH	GHIJ	GHI	GHI	A	ABCDE
11. IAA + Kinetin	HIJK	FGH	EFGH	HIJ	EFGH	BCDEF	A	ABC
12. IAA + ABA	EFGH	EFGH	DEFG	FGH	DEF	ABC	BCDEF	ABCDE
13. IAA + Ethrel	BC	A	BC	DEF	BCD	EFGHI	HIJ	E
14. GA ₃ + Kinetin	IJK	GHI	GH	J	J	JK	FAGI	BCDE
15. GA ₃ + ABA	DE	EFGH	DE	FGH	DE	AB	DEFGH	ABCDE
16. GA ₃ + Ethrel	CD	B	BC	CDE	DEFG	HI	HIJ	DE
17. Kinetin + ABA	L	I	H	IJ	FGH	BCDEFG	EFGHI	ABCDE
18. Kinetin + Ethrel	EF	C	C	B	B	BC	DEFGH	ABCDE
19. ABA + Ethrel	A	A	BC	BC	CD	EFGHI	GHIJ	CDE
20. Ethrel 500 ppm	AB	A	B	BCD	BCD	BCDEFGH	FAGI	CDE
21. ACT.-D 2.5 mg/l	HIJK	J	I	K	K	CDEFGHI	J	CDE
22. C - H 1 mg/l	EFG	CD	A	A	A	A	EFGHI	E
23. Control (water)	KL	HI	EDFGH	GHI	EFGH	ABC	BCDEF	ABCDE

¹ Average of 3 replications with 10 fruits per sample.² Means with the same letter are not significantly different at K=100 ($\frac{1}{2}$ 5% level).

Table 3. Ethylene evolution at 21° C of 'Floradel' tomato fruits treated with plant growth regulator and protein synthesis inhibitors.

Treatments	Days after harvest							
	1	2	3	4	5	6	7	8
	μl/kg/hr							
1. IAA 5mM	2.45 ¹	1.92	1.82	2.31	3.61	4.62	5.47	4.20
2. IAA 5x10 ⁻² mM	2.80	2.14	2.92	3.44	3.72	4.23	4.95	4.18
3. IAA 5x10 ⁻⁴ mM	2.27	2.07	2.98	3.29	3.76	4.22	5.01	4.24
4. GA ₃ 3mM	1.64	0.98	1.32	1.67	1.97	2.26	2.83	3.59
5. GA ₃ 3x10 ⁻⁴ mM	1.49	1.40	1.67	2.30	3.43	3.54	4.32	5.11
6. Kinetin 5 mM	1.53	1.15	1.08	1.23	2.20	3.35	3.96	5.30
7. Kinetin 5x10 ⁻⁴ mM	1.20	1.14	1.70	2.37	2.79	3.71	4.18	5.41
8. ABA 2mM	3.11	3.10	4.40	6.83	6.06	5.40	5.07	4.60
9. ABA 2x10 ⁻² mM	2.60	1.93	3.31	4.11	6.66	6.10	5.45	4.61
10. IAA+GA ₃	2.00	1.19	1.79	2.24	3.67	4.14	5.09	4.41
11. IAA+Kinetin	1.55	0.99	1.27	2.87	3.38	4.02	5.03	4.52
12. IAA+ABA	2.41	2.25	2.83	3.82	4.64	5.68	5.42	5.12
13. IAA+Ethrel	5.56	14.21	10.49	9.24	7.23	6.83	5.23	4.62
14. GA ₃ +Kinetin	1.57	0.87	1.43	1.25	2.74	3.20	3.65	4.44
15. GA ₃ +ABA	2.25	2.49	3.45	4.75	5.50	6.03	4.09	3.49
16. GA ₃ +Ethrel	4.30	13.12	8.51	6.24	5.13	4.69	4.42	4.09
17. Kinetin+ABA	2.70	1.26	2.00	2.66	3.54	4.77	4.29	3.52
18. Kinetin+Ethrel	4.61	6.12	7.45	11.73	7.63	5.56	4.93	4.55
19. ABA+Ethrel	6.93	13.80	10.78	9.21	6.74	6.21	5.72	5.13
20. Ethrel 500 ppm	5.69	14.62	11.12	9.37	7.86	6.32	5.91	5.03
21. Act.-D 2.5 mg/l	5.57	4.55	3.89	3.35	2.60	2.12	1.91	1.62
22. C-H 1 mg/l	3.02	4.25	8.75	5.47	4.34	3.94	3.54	3.06
23. Control (water)	2.02	1.98	2.87	4.30	4.58	5.27	4.61	4.40
LSD	1.05	1.98	1.74	1.75	1.46	1.56	1.48	1.13

Table 3 - continued (Waller-Duncan K ratio t-test).

Treatments	Days after Harvest							
	1	2	3	4	5	6	7	8
1. IAA 5mM	EFGH ²	DE	EFG	HIJ	HI	DEFGHI	ABC	BCD
2. IAA 5×10^{-2} mM	DEF	DE	CDEFG	EFGH	FGH	EFGHI	ABCDEF	BCDE
3. IAA 5×10^{-4} mM	DEFG	DE	CDEFG	EFGHIJ	FGH	EFGHI	ABCDEF	BCD
4. GA ₃ 3mM	GH	E	G	IJ	I	JK	GH	DE
5. GA ₃ 3×10^{-4} mM	GH	DE	EFG	HIJ	GHI	HIJK	BCDEF	ABC
6. Kinetin 5 mM	GH	E	G	J	HI	HIJK	DEFG	AB
7. Kinetin 5×10^{-4} mM	H	E	EFG	GHIJ	HI	GHIJ	CDEFG	A
8. ABA 2mM	DEFG	CD	C	CD	BCD	ABCDEF	ABCDE	ABCD
9. ABA 2×10^{-4} mM	DEF	DE	CDEF	DEFG	ABC	ABCD	ABC	ABCD
10. IAA+GA ₃	FGH	E	EFG	HIJ	FGH	EFGHI	ABCDE	ABCD
11. IAA+Kinetin	GH	E	G	FGHIJ	FGHI	FGHI	ABCDE	ABCD
12. IAA+ABA	DEFG	DE	CDEFG	EFGHI	EFG	ABCDE	ABCD	ABC
13. IAA+Ethrel	B	A	AB	B	AB	A	ABCD	ABCD
14. GA ₃ +Kinetin	GH	E	FG	J	HI	IJK	EFG	ABCD
15. GA ₃ +ABA	DEFG	DE	CDE	DE	CDE	ABCD	CDEFG	DE
16. GA ₃ +Ethrel	C	A	B	C	DEF	CDEFGHI	BCDEF	CDE
17. Kinetin+ABA	DEF	E	EFG	FGHIJ	GH	BCDEFGH	CDEFG	DE
18. Kinetin+Ethrel	BC	B	B	A	A	ASCDEF	ARCDEF	ABCO
19. ABA+Ethrel	A	A	A	B	ABC	ABC	AB	ABC
20. Ethrel 500 ppm	B	A	A	B	A	AB	A	ABC
21. Act.-D 2.5 mg/l	B	BC	CD	EFGHI	HI	K	H	F
22. C-H 1 mg/l	DE	C	B	CD	EFG	GHI	FG	E
23. Control (water)	CFGH	E	CDEFG	DEF	EFG	ABCDEFG	ABCDEF	ABCD

¹ Average of 3 replications with 10 fruits per sample.

² Means with the same letter are not significantly different at K=100 (1% probability level).

Table 4. Color development¹ of 'Floradel' tomato treated with plant growth regulator and protein-synthesis inhibitors at mature-green stage, then held at 21° C.

Treatments	Days after harvest		
	2	4	6
1. IAA 5mM	14.00 ² BC0 ³	22.00 IJ	42.00 G
2. IAA 5×10^{-2} mM	10.00 OEF	26.00 GHIJ	42.00 G
3. IAA 5×10^{-4} mM	10.00 OEF	26.00 GHIJ	46.00 EF
4. GA ₃ 3mM	6.00 FGH	22.00 IJ	28.00 K
5. GA ₃ 3×10^{-4} mM	2.67 GH	24.00 HIJ	30.00 JK
6. Kinetin 5mM	8.00 EFG	26.00 GHIJ	35.00 HI
7. Kinetin 5×10^{-4} mM	8.00 EFG	28.00 FGHI	36.00 HI
8. ABA 2mM	8.00 EFG	40.00 COE	56.00 C-
9. ABA 2×10^{-4} mM	10.67 COEF	36.00 OEF	52.00 O
10. IAA+GA ₃	6.00 FGH	26.00 GHIJ	36.00 HI
11. IAA+Kinetin	6.00 FGH	28.00 FGHI	34.00 I
12. IAA+Ethrel	10.00 AB	32.00 EFGH	55.00 CO
13. IAA+Ethrel	18.00 AB	47.33 ABC	64.00 B
14. GA ₃ +Kinetin	6.00 FGH	24.00 HIJ	34.00 I
15. GA ₃ +ABA	12.00 COE	34.00 DEFG	48.00 E
16. GA ₃ +Ethrel	16.00 ABC	42.00 BCO	52.00 O
17. Kinetin+ABA	10.00 OEF	32.00 EFGH	46.00 EF
18. Kinetin+Ethrel	20.00 A	46.00 ABC	58.00 C
19. ABA+Ethrel	20.00 A	54.00 A	71.33 A
20. Ethrel 500 ppm	18.00 A	30.00 A	70.00 A
21. ACT.-0 2.5 mg/l	6.67 EFGH	31.33 EFGH	33.33 JL
22. C-H 1 mg/l	1.33 H	16.33 J	16.67 L
23. Control (water)	10.00 OEF	30.00 FGHI	44.00 FG
LSO	5.493	9.883	3.848

¹ 0=mature-green, 20=breaker, 40=turning, 60=pink, 80=red.

² Average of 3 replications with 20 fruits per sample.

³ Means with the same letter are not significantly different at K=100 (± 5% probability level).

Data was tested with Waller-Duncan k ratio t-test.

Table 5. Ethylene evolution at 21° C of 'Rin' tomato treated with plant growth regulators and protein-synthesis inhibitors.

Treatments	Days after harvest							
	1	2	3	4	5	6	7	8
	$\mu\text{ l/kg/day}$							
1. IAA 5 mM	0.34 ¹	0.43	0.08	0.28	0.24	0.11	0.90	0.89
2. IAA 5×10^{-2} mM	0.38	0.12	0.43	0.77	0.29	0.29	0.62	0.49
3. IAA 5×10^{-4} mM	0.23	0.84	0.69	0.34	0.55	0.41	0.61	0.33
4. GA ₃ 3 mM	0.21	0.28	0.37	0.88	0.89	0.70	0.49	0.26
5. GA ₃ 3×10^{-4} mM	0.13	0.18	0.22	0.57	0.98	0.87	0.57	0.78
6. Kinetin 5 mM	0.46	0.98	0.19	0.78	0.90	0.52	0.51	0.65
7. Kinetin 5×10^{-4} mM	0.70	0.19	0.22	0.77	0.37	0.42	0.50	0.61
8. ABA 2mM	0.13	0.62	0.67	0.85	0.48	0.79	0.79	0.49
9. ABA 2×10^{-4} mM	0.17	0.31	0.81	0.68	0.32	0.24	0.33	0.59
10. IAA + GA ₃	0.28	0.52	0.57	0.47	0.21	0.78	0.79	0.41
11. IAA + Kinetin	0.44	0.16	0.53	0.67	0.12	0.20	0.21	0.71
12. IAA + ABA	0.38	0.20	0.16	0.69	0.34	0.68	0.51	0.79
13. IAA + Ethrel	0.52	0.89	0.74	0.15	0.70	0.79	0.25	0.23
14. GA ₃ + Kinetin	0.27	0.85	0.25	0.61	0.71	0.75	0.64	0.41
15. GA ₃ + ABA	0.33	0.81	0.79	0.23	0.39	0.68	0.59	0.11
16. GA ₃ + Ethrel	0.66	0.31	0.44	0.87	0.50	0.90	0.89	0.20
17. Kinetin + ABA	0.49	0.12	0.28	0.80	0.78	0.63	0.40	0.11
18. Kinetin + Ethrel	0.41	0.10	0.69	0.79	0.61	0.39	0.31	0.77
19. ABA + Ethrel	0.17	0.62	0.31	0.78	0.50	0.76	0.29	0.10
20. Ethrel 500 ppm	0.49	0.79	0.60	0.83	0.27	0.61	0.46	0.39
21. C-H 2.5 mg	0.39	0.53	0.48	0.79	0.80	0.38	0.00	0.00
22. Act.-D 1 mg/l	0.15	0.23	0.45	0.42	0.58	0.26	0.29	0.20
23. Control (water)	0.33	0.58	0.60	0.22	0.81	0.33	0.49	0.45

Harvest Day = July 25, 1976.

¹ Rate per 5=fruit sample.

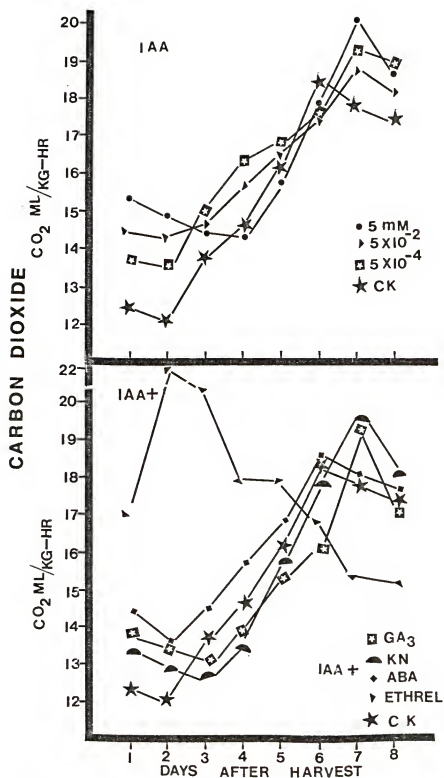


Figure 6. Influence of IAA treatments on respiration rate of 'Floradel' tomato fruits at 21° C.

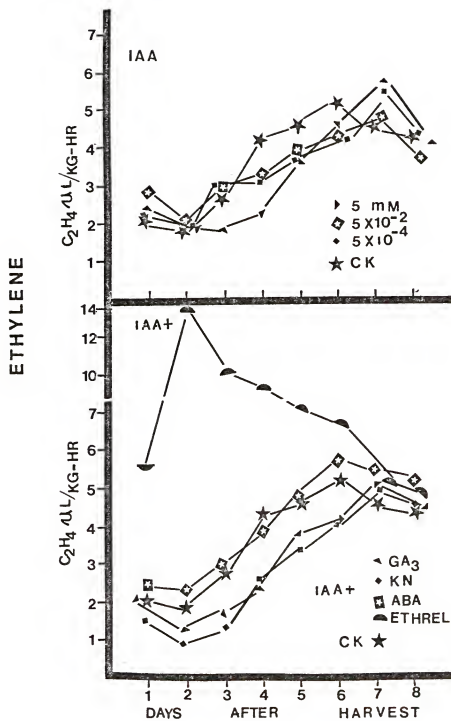


Figure 7. Influence of IAA treatments on ethylene evolution of 'Floradel' tomato fruits at 21° C.

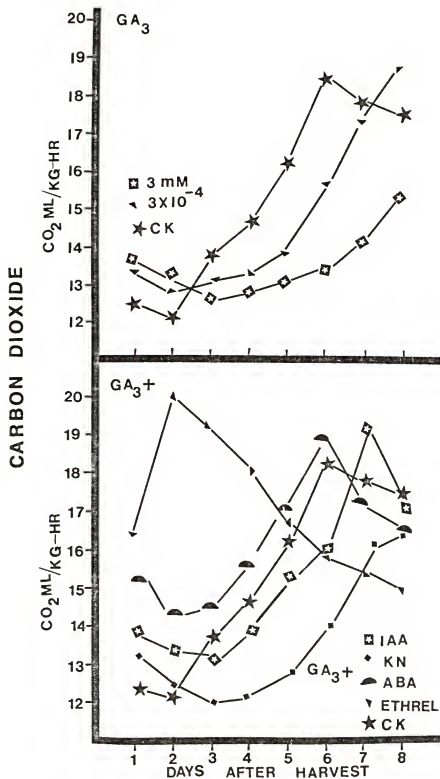


Figure 8. Influence of gibberellin A₃ treatments on respiration rate of 'Floradel' tomato fruits at 21° C.

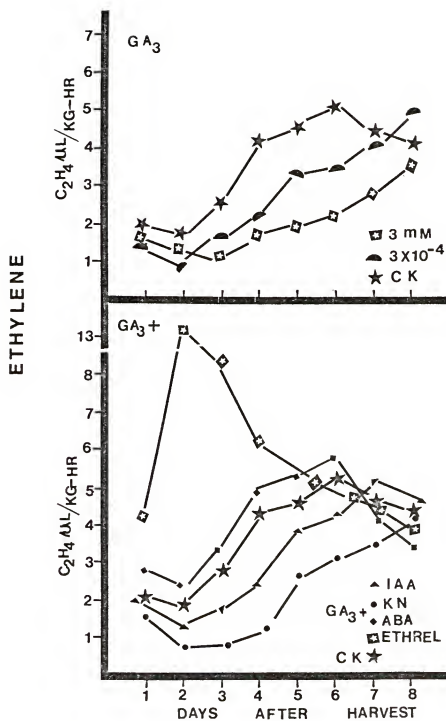


Figure 9. Influence of gibberellin A₃ treatments on ethylene evolution of 'Floradel' tomato fruits at 21° C.

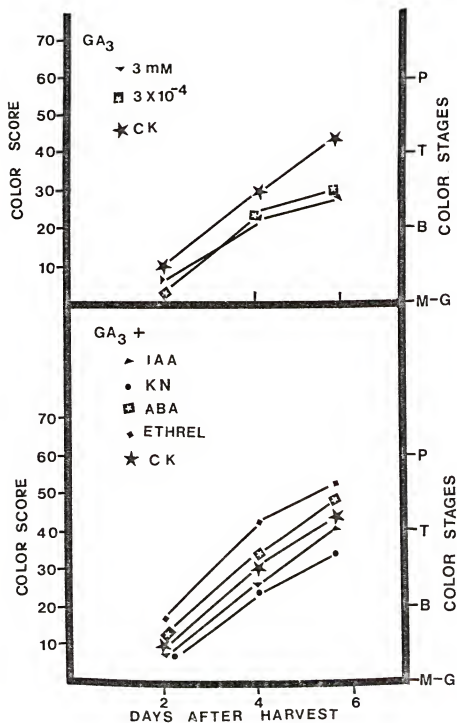


Figure 10. Influence of gibberellin A₃ treatments on color development of 'Floradel' tomato fruits at 21°C.

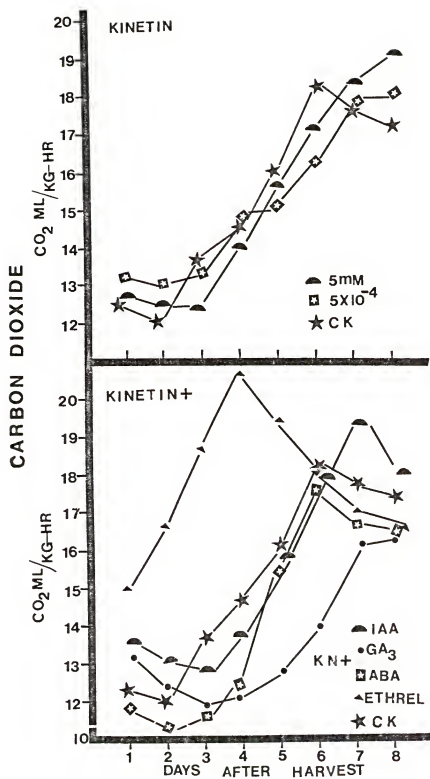


Figure 11. Influence of Kinetin treatments on respiration rate of 'Floradel' tomato fruits at 21° C.

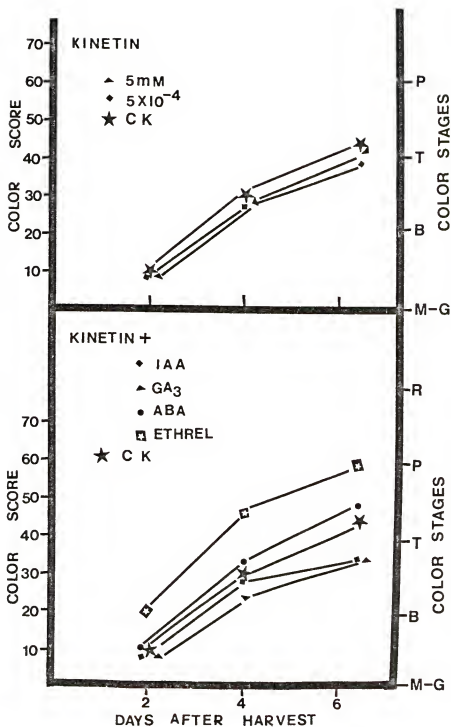


Figure 12. Influence of Kinetin treatments on ethylene evolution of 'Floradel' tomato fruits at 21° C.

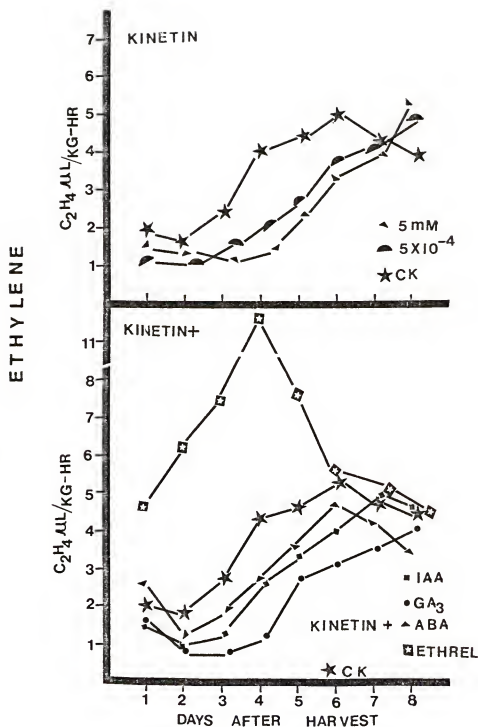


Figure 13. Influence of Kinetin treatments on color development of 'Floradel' tomato fruits at 21° C.

CARBON DIOXIDE

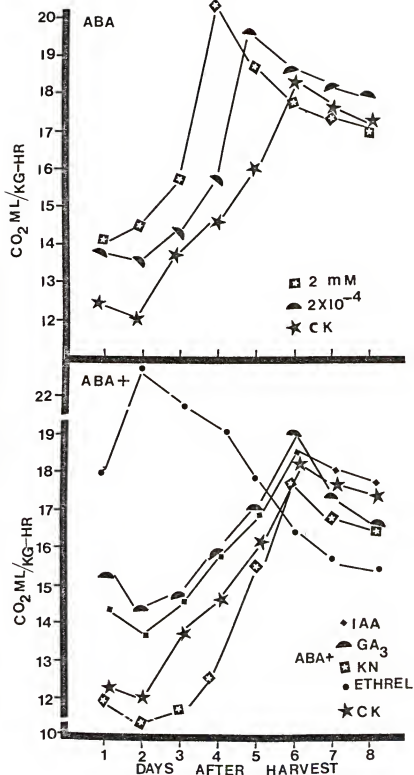


Figure 14. Influence of abscisic acid treatments on respiration rate of 'Floradel' tomato fruits at 21° C.

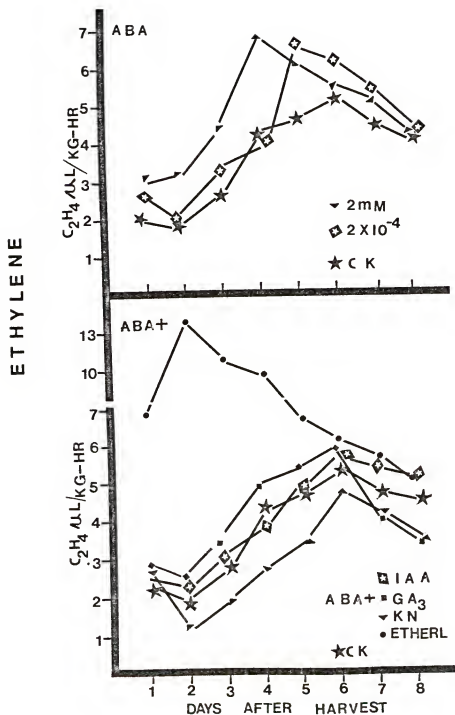


Figure 15. Influence of abscisic acid treatments on ethylene evolution of 'Floradel' tomato fruits at 21° C.

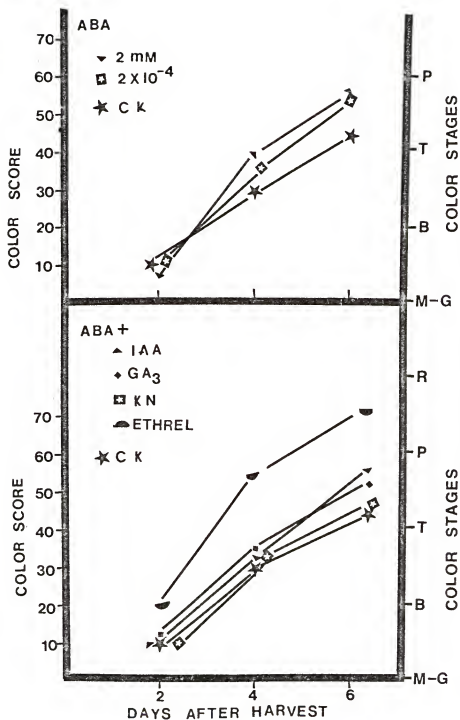


Figure 16. Influence of abscisic acid treatments on color development of 'Floradel' tomato fruits at 21° C.

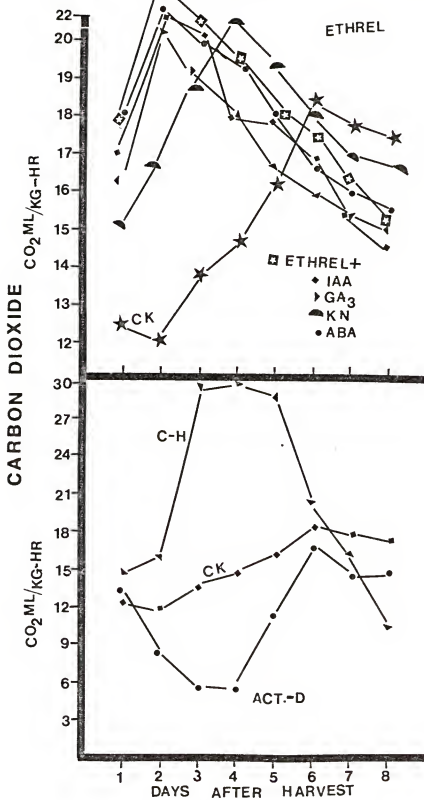


Figure 17. Influence of protein synthesis inhibitors and Ethrel treatments on respiration rate of 'Floradel' tomato fruits at 21° C.

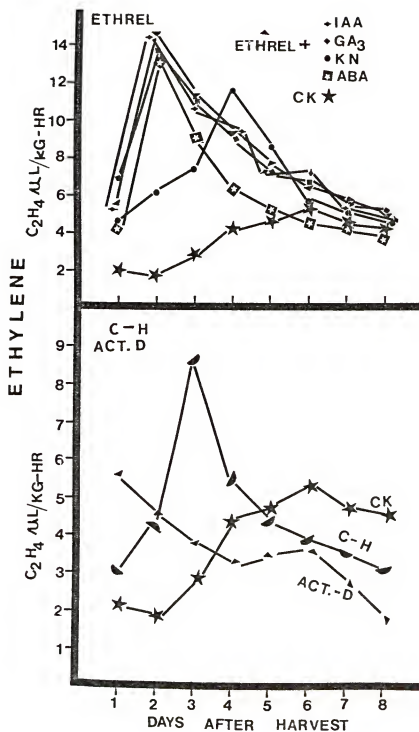


Figure 18. Influence of protein synthesis inhibitors and Ethrel treatments on ethylene evolution of 'Floradel' tomato fruits at 21° C.

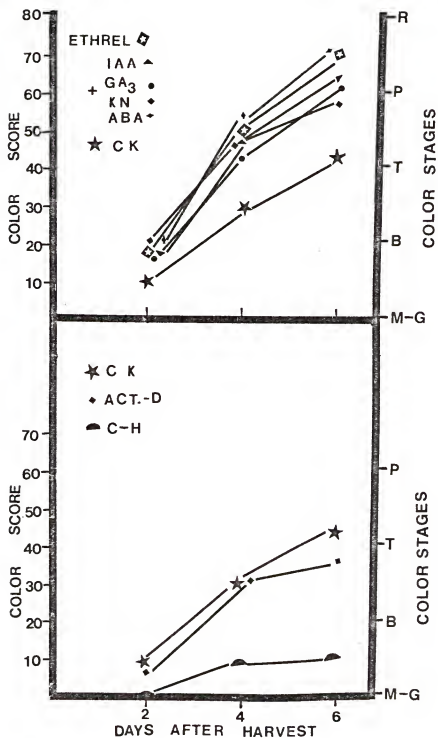


Figure 19. Influence of protein synthesis inhibitors and Ethrel treatments on color development of 'Floradel' tomato fruits at 21° C.

increased respiration rate for 3 days and actinomycin D decreased it. Cycloheximide had a phytotoxic effect on both 'Floradel' and 'Rin' tomatoes expressed in respiration which unusually increased after treatment (Table 2, 6).

IAA and its combinations. Fruits treated with IAA concentration between 5×10^{-4} to 5 mM had a higher respiration rate during the first 3 days. The time to initiate the climacteric rise was inversely proportional to the concentration of IAA (Table 2). There was a one-day delay in attainment of the climacteric peak compared to the control (Fig. 7, upper). Changes of ethylene evolution (Fig. 4 upper) were similar to respiration rate. Color development appeared slightly delayed in the fruits treated with IAA, but this delay was not significant. The 5 mM treatment suppressed color development during the first 4 days (Fig. 6, upper).

Respiration rate (Fig. 7) and ethylene evolution (Fig. 5) as a result of IAA+Ethrel treatment increased abruptly during 48 hrs after treatment but then decreased sharply for the next two days after the climacteric peak. The decrease continued gradually until the 7th day. Fruit color showed breaker, turning and pink stage at 2, 4, and 6 days, respectively (Fig. 6, lower). Treatment of IAA+ABA resulted in a slightly higher respiration rate and ethylene evolution but otherwise compared favorably to the control. Treatments of IAA+Kinetin and IAA+GA₃, caused a one-day delay in initiation and attaining the climacteric rise (Fig. 5, 7, lower). Fruit color in these two treatments was lower than the control during color development (Fig. 2, lower).

Table 6. Respiration rate at 21° C of 'Rin' tomatoes treated with plant growth regulators and protein-synthesis inhibitors.

Treatments	Days after harvest							
	1	2	3	4	5	6	7	8
	CO ₂ ml/kg/hr							
1. IAA 5 mM	16.67 ¹	15.54	14.32	13.60	13.54	13.74	13.97	13.57
2. IAA 5x10 ⁻² mM	17.10	16.42	15.74	14.95	14.30	13.89	13.94	14.10
3. IAA 5x10 ⁻⁴ mM	17.00	16.68	15.23	14.55	13.95	14.40	13.50	13.66
4. GA ₃ 3 mM	16.43	15.50	14.80	13.88	14.32	13.69	14.37	13.92
5. GA ₃ 3x10 ⁻⁴ mM	17.32	16.40	15.53	14.83	14.50	14.13	14.87	13.45
6. Kinetin 5 mM	15.64	15.75	15.00	14.60	14.28	13.22	13.60	14.05
7. Kinetin 5x10 ⁻⁴ mM	16.27	15.70	14.95	13.77	13.71	12.41	14.17	16.93
8. ABA 2 mM	16.16	15.40	14.29	13.28	13.16	13.51	13.78	13.99
9. ABA 2x10 ⁻⁴ mM	15.13	14.64	14.19	13.30	13.34	13.65	13.72	12.74
10. IAA + GA ₃	15.79	14.94	13.55	12.85	13.29	13.64	12.52	12.63
11. IAA + Kinetin	16.48	15.21	13.90	12.01	12.70	13.47	13.04	13.83
12. IAA + ABA	17.68	16.79	15.37	13.44	13.73	14.18	13.99	13.03
13. IAA + Ethrel	16.97	18.70	17.83	15.99	14.89	14.43	14.17	14.46
14. GA ₃ + Kinetin	15.89	14.13	13.74	12.82	12.27	12.81	13.33	14.38
15. GA ₃ + ABA	16.31	15.21	14.20	13.74	13.96	13.79	12.55	12.71
16. GA ₃ + Ethrel	17.43	19.21	18.15	16.37	15.11	14.87	13.12	13.87
17. Kinetin + ABA	16.44	15.12	14.40	13.61	12.69	13.93	12.72	13.62
18. Kinetin + Ethrel	17.08	19.74	18.78	16.30	15.48	13.50	12.53	13.83
19. ABA + Ethrel	17.61	18.82	19.29	16.21	14.98	13.95	13.02	13.34
20. Ethrel 500 ppm	17.44	19.90	18.24	16.88	15.65	14.69	13.54	13.30
21. C-H 2.5 mg/l	16.83	18.37	19.73	21.74	23.56	16.58	10.13	5.74
22. Act.-D 1 mg/l	16.62	14.13	12.15	10.74	9.68	9.11	7.74	7.36
23. Control (water)	16.74	15.08	14.62	13.79	13.50	13.27	13.54	13.37

Harvest Day = July 25, 1976.

¹ Rate per 5-fruit sample.

GA₃ and its combinations. Fruits treated with gibberellin A₃ at concentration of 3 mM delayed about one day the initiation of climacteric rise both in respiration rate and ethylene evolution. The increased slope was more gradual and did not exhibit the climacteric peak 8 days after treatment (Fig. 8, 9, upper). Fruit color developed more slowly and reached a color score of 28 in 6 days as compared to a color score of 44 for the control (Fig. 10, upper).

Treatment with GA₃+Ethrel resulted in the attainment of the climacteric peak in 48 hrs after treatment. Thereafter, there was a gradual decrease in respiration and ethylene evolution until the 8th day (Fig. 8, 9, lower). However, color development did not proceed as rapidly as respiration, whereas ethylene evolution did (Fig. 10, lower). Treatment with GA₃+ABA caused a higher but similar change in respiration rate, ethylene evolution, and color development compared with the control (Fig. 8, 9, 10). Treatments of GA₃+IAA and GA₃+Kinetin resulted in a slightly higher respiration rate but lower ethylene evolution during the initial 48 hours. Thereafter the increase was slow until the climacteric peak was reached. As compared to the control, GA₃+IAA delayed attainment of the peak by one day while GA+Kinetin caused non-attainment of the climacteric peak even after 8 days. Ethylene evolution changes were somewhat correlated to the respiration changes; however, GA₃+IAA initiated the climacteric (ethylene evolution) one day earlier than the respiration rise. Treatment of GA₃+ABA caused a higher respiration rate and ethylene evolution which then decreased sharply during the post-climacteric period (Fig. 8, 9, lower). The highest rate in color development among the combinations resulted from treatment with GA₃+Ethrel. At the 6th day, this treatment resulted in a color score of

52 as compared to the control with a color score of 44 and GA_3 +Kinetin at 34 (Fig. 10, lower).

Kinetin and its combinations. Fruits treated with Kinetin showed a higher initial respiration rate during the first 2 days but the initiation of climacteric rise was delayed one day. Thereafter, respiration was comparable to that for the control (Fig. 11, 12, upper). After 6 days of ripening, Kinetin treated fruit had a color score of 35 as compared to 44 for the control (Fig. 13, lower).

As a result of the Kinetin+Ethrel treatment, respiration rate and ethylene evolution increased sharply and reached the climacteric peak after 4 days; postclimacteric decline was similar compared to the control until the 8th day. Kinetin+ABA caused a very low respiration rate in the preclimacteric dip, but the rate then increased similarly to the control. Kinetin+ GA_3 resulted in a low respiration rate and ethylene evolution which did not attain a climacteric peak in the 8-day period. Kinetin+IAA retarded for one day the initiation of climacteric rise in respiration but not in ethylene (Fig. 11, 12, lower).

Fruits in the Kinetin+Ethrel treatment developed to a color score of 20 (breaker) by the 2nd day, over 40 (turning) by the 4th day and reached 60 (pink) at the 6th day. Kinetin+ABA fruit had a color score of 46 as compared to 44 for the control at the 6th day. Kinetin+ GA_3 showed the similar rate of color development compared to control, however, the rate increased slowly between the period of 4-6 days; Kinetin+ GA_3 had the lowest color development among all combinations (Fig. 13, lower).

ABA and its combinations. Fruit treated with ABA had an increase in both respiration and ethylene as compared to the control. The increase was abrupt and attained the climacteric peak at days 4 and 5 with the concentrations of 2 mM and 2×10^{-4} mM, respectively (Fig. 14, 15, upper). Fruit color developed sooner in ABA treatment; a pink color stage was reached in ABA treatment after 6 days while the control was only at the turning stage (Fig. 16, upper).

Treatment of ABA+Ethrel resulted in a substantially increased respiration rate and ethylene evolution attaining the climacteric peak within 48 hrs. Thereafter, there was a gradual decline until the 7th day. Treatment with ABA+IAA and ABA+GA₃ caused a higher respiration rate and ethylene evolution than the control, while ABA+Kinetin lowered the rate prior to the preclimacteric peak (Fig. 14, 15, lower).

Abscisic acid+Ethrel treatment accelerated color development; the pink stage was reached by the 4th day and the red stage by the 6th day. Color development as a result of ABA+IAA and ABA+GA₃ was only about one half color stage higher than the control after 6 days ripening. Treatment with ABA+Kinetin developed at a similar rate as compared to the control (Fig. 16, lower).

Ethrel and its combinations. Respiration and ethylene evolution proceeded at a very rapid rate and attained the climacteric peak within 48 hours as a result of all treatments except Ethrel+Kinetin. Decline after the peak was gradual until the 8th day. Fruits treated with Ethrel+Kinetin attained their climacteric peak on the 4th day after treatment (Fig. 17, 18).

Color development was very rapid as a result of Ethrel treatments, particularly between the 2nd and 6th days after treatment. The exception

again, was Ethrel+Kinetin. After 6 days, fruit from most treatments had reached the pink stage as compared to the control which was only at the turning stage (Fig. 19, upper).

Protein synthesis inhibitors. Treatment with cycloheximide was phytotoxic to the fruit. Within 4 days after treatment a deterioration of tissue occurred around the stem scar. Data on color, respiration, and ethylene evolution are invalid because of the advanced stage of decay.

Fruits treated with actinomycin D had a sharply decreased respiration rate until the 4th day after which the climacteric peak was quickly attained. Ethylene evolution also decreased sharply after which there was a slight increase until the climacteric peak. The maximum peak both in respiration rate and ethylene evolution were quite low compared to the control. Fruit color resulting from the actinomycin D treatment developed normally and at a rate similar to the control for the first 4 days; color development was less rapid thereafter (Fig. 17, 18, 19, lower).

Protein Content and Enzyme Activity

Partial Purification of Enzyme Extracts

Data on partial purification of peroxidase and IAA oxidase in tomato extracts are shown in Table 7. Tomato extracts purified with ammonium sulfate fractionation showed high protein content between 30-40% saturation, but was reduced at a saturation higher than 50%. Total activity and specific activity both in peroxidase and IAA oxidase was very low in the fraction under 50% saturation. Most peroxidase and IAA oxidase activity existed in the fractions between 50-80% saturation.

Table 7. Activities of peroxidase and IAA oxidase from tomato enzyme extracts partially purified by ammonium sulfate fractionation and dialysis in phosphate buffer.

Saturation	Protein content mg/g f.w.	Peroxidase		IAA Oxidase	
		Total activity	Specific activity	Total activity	Specific activity
%		units ³ x 10 ⁻²	units/mg protein	units ⁴	units/mg protein
(0)	0.120 $\pm$ 0.044 ²	27.6 $\pm$ 2.72	2.30 $\pm$ 0.42	0.09 $\pm$ 0.008	0.82 $\pm$ 0.22
0-20	0.0217 $\pm$ 0.0031	17.5 $\pm$ 2.14	8.99 $\pm$ 0.97	0.52 $\pm$ 0.006	23.96 $\pm$ 2.84
20-30	0.0157 $\pm$ 0.0015	6.3 $\pm$ 0.67	4.01 $\pm$ 0.60	0.51 $\pm$ 0.020	32.00 $\pm$ 2.51
30-40	0.0317 $\pm$ 0.0010	7.1 $\pm$ 0.88	2.24 $\pm$ 0.22	0.50 $\pm$ 0.062	15.77 $\pm$ 2.23
40-50	0.0111 $\pm$ 0.0013	16.3 $\pm$ 0.11	14.69 $\pm$ 1.68	0.50 $\pm$ 0.072	45.05 $\pm$ 11.38
50-60	0.0073 $\pm$ 0.0026	146.7 $\pm$ 39.1	195.60 $\pm$ 94.92	5.87 $\pm$ 0.877	782.67 $\pm$ 267.39
60-70	0.0092 $\pm$ 0.0025	94.0 $\pm$ 11.12	102.17 $\pm$ 44.68	5.82 $\pm$ 0.890	632.61 $\pm$ 316.58
70-80	0.0068 $\pm$ 0.0046	82.7 $\pm$ 6.47	121.62 $\pm$ 16.08	5.77 $\pm$ 0.147	848.53 $\pm$ 76.27
80-90	0.0073 $\pm$ 0.0015	27.7 $\pm$ 2.09	37.95 $\pm$ 10.28	0.66 $\pm$ 0.077	90.26 $\pm$ 24.64

¹ Average of 3 replications with 3 fruits per sample.

² Standard deviation.

³ One unit was defined as decomposing 1 μ mole of H₂O₂ per min at 25° C.

⁴ One unit was defined as 1 μ g IAA destroyed in 30 min at 35° C.

Enzyme Characteristics

Enzyme extracts were partially purified with ammonium sulfate. The fractionation between 60-70% saturation was used to study the enzyme characteristics.

Dilution and incubation test. Purified enzyme extract diluted from 5 to 1,000 fold show enzyme activity in terms of amount of IAA destroyed in the enzyme reaction mixture. There was a linear regression relationship between dilution and enzyme activity with a correlation coefficient $r=-0.93$; however, the specific activity of IAA oxidase showed an increase until up to 500 fold dilution (Table 8). The incubation time and enzyme activity also showed a linear regression relationship in 40 min with a correlation coefficient $r=-0.94$ (Table 9).

Induction effect. Indoleacetic acid in a reaction mixture (15 $\mu\text{g/ml}$) was 70% destroyed in 30 min by a partially purified enzyme extract. Between 30 and 45 min, destruction was stable at a low level with only slight activity at the end of the period. When the same amount of IAA was again added into the previous reaction mixture in the starvation stage, a similar enzyme activity was again obtained (Fig. 20).

Comparison of Protein Content and Peroxidase Activity by 'Floradel' and 'Rin' Tomato at Designated Stages of Color Development

There was no difference in protein content due to either cultivar or stage of color development (Table 10). Peroxidase total activity in 'Floradel' tomato increased about 3 times from green to breaker stage, increased slightly to the turning stage and then decreased at the pink stage. However, peroxidase activity was not associated with color stage in 'rin' tomatoes, although enzyme activity was slightly

Table 8. Effect of dilution on IAA oxidation by tomato enzyme extracts between 60-70% ammonium sulfate saturation and dialysis in phosphate buffer.

Dilution	Protein content	O.D.	IAA content	IAA destroyed	Specific activity ²
	µg/g/f.w.	530 mµ	µg/cc	µg/cc	unitsx10 ³ /mg protein
<u>X</u>				<u>Y</u>	<u>Z</u>
5	1.850 ¹	0.125	1.121	16.370	8.849
9	1.022	0.155	1.726	15.780	15.440
50	0.184	0.253	3.702	13.998	76.076
100	0.092	0.338	5.405	12.100	131.522
150	0.061	0.470	8.077	9.423	154.475
250	0.037	0.495	8.581	8.919	231.919
500	0.018	0.665	12.010	5.490	305.000
1000	0.0092	0.845	15.640	1.863	202.500

Linear regression $X = 13.948 - 0.0138 Y$; $x = 90.335 + 0.1997 Z$

Correlation coefficient $r = -0.9293$; $r_{xz} = 0.649$

¹ Average of two replications with 3 fruits per sample.

² One unit was defined as 1 µg IAA destroyed in 30 min at 35° C.

Table 9. Effect of incubation time on the oxidation of IAA by tomato enzyme extract between 60-70% ammonium sulfate saturation and dialysis in phosphate buffer.

Incubation time	O.D.	IAA content	IAA destroyed
min	530 mμ	μg/ml	μg/ml
<u>X</u>			<u>Y</u>
0	0.92 ¹	17.500	---
5	0.77	14.290	3.210
10	0.68	12.310	5.190
15	0.51	8.883	8.617
20	0.35	5.657	11.843
25	0.32	5.052	12.448
30	0.28	4.246	13.256
35	0.22	3.306	14.464
40	0.19	2.431	15.069

Linear regression $Y = 6.976 + 0.305 X$

Correlation coefficient $r_{xy} = 0.9407$

¹ Average of two replications with 3 fruits per sample.

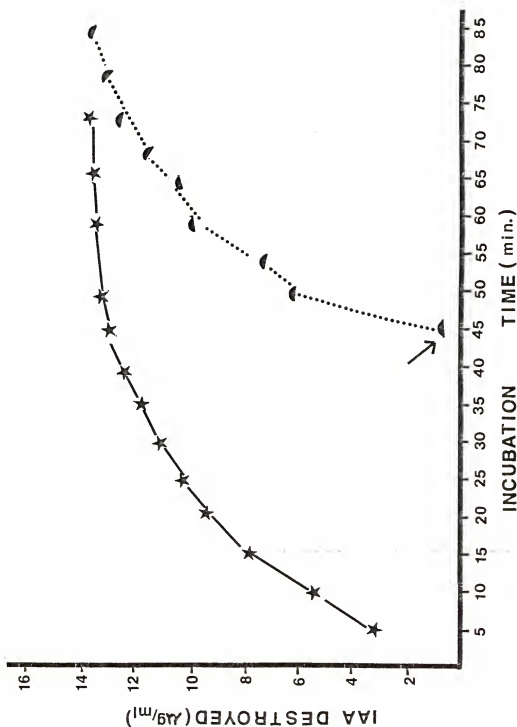


Figure 20. Induction effect after cessation of IAA oxidation by tomato enzyme extracts partially purified at 60-70% ammonium sulfate saturation and dialysis in phosphate buffer. At the arrow, 15 $\mu\text{g}/\text{ml}$ IAA was reapplied.

Table 10. Comparisons of protein content and peroxidase activity of 'Floradel' and 'Rin' tomato at designated stages of color development.

Color stages	Protein content	Total activity	Specific activity
	mg/g f.w.	units	units/mg protein
Floradel ¹			
Green	0.0240±0.0028 ³	3.320±0.622	139.067±21.956
Breaker	0.0248±0.0026	8.913±1.536	355.055±51.187
Turning	0.0242±0.0040	8.756±1.292	368.153±77.198
Pink	0.0245±0.0038	7.469±1.188	316.169±74.057
Rin ²			
Green	0.0286±0.0084	4.865±1.392	174.81±10.952
Green-yellow	0.0241±0.012	4.530±1.084	186.95±38.238
Yellow-green	0.0244±0.029	4.173±0.574	171.24±11.105
Yellow	0.0246±0.049	4.579±1.074	185.43±18.584

¹ Average of 3 replications with 3 fruits per sample.

² Average of 2 replications with 2 fruits per sample.

³ Standard deviation.

higher at the green stage. Peroxidase specific activity showed similar changes as total activity in 'Floradel' tomato but there was no change in 'Rin' tomato when treated with plant growth regulators.

Effect of Plant Growth Regulators on Protein and Peroxidase Specific Activity of 'Floradel' Tomato Fruit at Designated Stages of Color Development

Results of these tests were analyzed using the Waller-Duncan Multiple Range Tests and data are shown in Table 11-14.

Growth regulators did not affect protein content during color development. The protein inhibitor α -cycloheximide strongly inhibited protein synthesis and decreased protein content in each color stage whereas actinomycin D had only inhibitive effects at the breaker stage. Graphic presentation of growth regulator effects upon peroxidase specific activity are shown in Fig. 21-25.

Peroxidase specific activity was promoted by IAA. The degree of promotion was proportional to concentration in mature-green and breaker stage but not in turning and pink stages. Combinations of IAA+Kinetin and IAA+GA₃ had no such effect. Treatment with IAA+ABA increased activity in mature-green and breaker stages but not in other stages as compared to the control (Fig. 21).

Fruits treated with GA₃ at concentration of 3 mM and 3×10^{-4} mM inhibited peroxidase activity at the mature-green and breaker stage. Combinations of GA₃+IAA and GA₃+Kinetin showed the same effect as GA₃ alone, whereas GA₃+ABA and GA₃+Ethrel exerted an opposite effect. Treatment with GA₃+Ethrel caused a sharply decreased peroxidase specific activity between the breaker and turning stages (Fig. 22).

Kinetin inhibited peroxidase specific activity more intensely and for a longer period of time than IAA and GA₃. There were no synergistic

Table 11. Protein content and peroxidase activity of 'Floradel' tomato treated with plant growth regulators and protein-synthesis inhibitors at mature-green stage, then held at 21° C.

Treatments	Protein content	Peroxidase	
		Total activity	Specific activity
	mg/g f.w.	units	units/mg protein
1. IAA 5mM	0.0232 ¹ AB ²	4.563 E	196.686 D
2. IAA 5x10 ⁻² mM	0.0211 BC	3.880 EFG	184.002 DE
3. IAA 5x10 ⁻⁴ mM	0.0232 AB	3.642 EFG	156.991 EF
4. GA ₃ 3mM	0.0280 A	2.109 HI	75.331 G
5. GA ₃ 3x10 ⁻⁴ mM	0.0257 AB	1.603 HIJ	64.012 G
6. Kinetin 5 mM	0.0236 AB	1.704 HIJ	72.333 GH
7. Kinetin 5x10 ⁻⁴ mM	0.0267 AB	2.165 HI	80.976 G
8. ABA 2 mM	0.0246 AB	3.802 EFG	153.003 EF
9. ABA 2x10 ⁻⁴ mM	0.0236 AB	3.580 EFG	156.003 EF
10. IAA+GA ₃	0.0258 AB	1.469 IJ	57.986 G
11. IAA+Kinetin	0.0265 AB	2.031 HIJ	76.989 G
12. IAA+ABA	0.0241 AB	3.533 FG	152.928 DF
13. EAA+Ethrel	0.0252 AB	6.357 C	256.010 C
14. GA ₃ +Kinetin	0.0239 AB	1.594 HIJ	67.012 G
15. GA ₃ +ABA	0.0237 AB	4.344 EF	184.672 DE
16. GA ₃ +Ethrel	0.0217 BC	5.952 CD	275.006 BC
17. Kinetin+ABA	0.0216 BC	1.767 GH	81.353 G
18. Kinetin+Ethrel	0.0219 B	5.332 DE	244.625 C
19. ABA+Ethrel	0.0261 AB	7.473 B	301.009 AB
20. Ethrel 500 ppm	0.0261 AB	8.635 A	334.247 A
21. C-H 2.5 mg/l	0.0159 C	1.042 J	65.995 G
22. ACT.-D 1 mg/l	0.0192 BC	2.574 GHI	133.39 F
23. Control (water)	0.0258 AB	3.301 G	128.002 F
LSD	0.0060	0.955	33.568

¹ Average of 3 replications with 2 fruits per sample.

² Means with the same letter are not significantly different at K=100 (½ 5% probability level).

Data was tested by Waller-Duncan k ratio t-test.

Table 12. Protein content and peroxidase activity of 'Floradel' tomato at breaker stage when treated with plant growth regulators and protein-synthesis inhibitors at mature-green stage, then held at 21° C.

Treatments	Protein content	Peroxidase	
		Total activity	Specific activity
	mg/g f.w.	units	units/mg protein
1. IAA 5mM	0.0284 ¹ ABC ²	10.905 FGH	383.996 CDE
2. IAA 5x10 ⁻² mM	0.0264 BCD	9.814 EFG	371.727 CDEF
3. IAA 5x10 ⁻⁴ mM	0.0233 DEF	7.586 GH	324.494 EFGH
4. GA ₃ 3 mM	0.0270 ABCD	8.035 FGH	296.123 FGH
5. GA ₃ 3x10 ⁻⁴ mM	0.0266 BCD	7.776 FGH	292.319 FGH
6. Kinetin 5 mM	0.0290 AB	8.758 DEFGH	300.925 FGH
7. Kinetin 5x10 ⁻⁴ mM	0.0271 ABCD	7.123 HI	263.238 H
8. ABA 2 mM	0.0260 BCD	10.914 BCDE	417.195 CD
9. ABA 2x10 ⁻⁴ mM	0.0286 ABC	12.192 BC	425.996 CD
10. IAA+GA ₃	0.0287 AB	9.143 DEFGH	313.670 EFGH
11. IAA+Kinetin	0.0308 A	10.142 CDEF	328.399 EFGH
12. IAA+ABA	0.0206 EFG	8.153 FGH	394.866 CDE
13. IAA+Ethrel	0.0253 BCD	10.900 BCDE	429.568 CD
14. GA-Kinetin	0.0257 BCD	7.288 HI	282.852 GH
15. GA+ABA	0.0188 GH	8.565 EFGH	444.758 BC
16. GA+Ethrel	0.0288 AB	12.675 AB	439.366 BCD
17. Kinetin+ABA	0.0258 BCD	8.298 FGH	319.797 EFGH
18. Kinetin+Ethrel	0.0267 BCD	11.118 BCD	412.404 CD
19. ABA+Ethrel	0.0254 BCD	14.679 A	576.923 A
20. Ethrel 500 ppm	0.0244 CDE	12.548 AB	514.746 AB
21. C-H 2.5 mg/l	0.0132 I	2.708 J	130.920 I
22. Act.-D 1 mg/l	0.0162 HI	5.683 I	250.85 EDFG
23. Control (water)	0.0261 BCD	9.392 EFGH	359.384 EDFG
LSD	0.00431	2.478	82.535

¹ Average of 3 replications with 2 fruits per sample.

² Means with the same letter are not significantly different at K=100 (± 50% probability level).

Data was tested by Waller-Duncan k ratio t-test.

Table 13. Protein content and peroxidase activity of 'Floradel' tomato at turnign stage when treated with plant growth regulators and actinomycin D at mature-green stage, then held at 21° C.

Treatments	Protein content	Peroxidase	
		Total activity	Specific activity
	mg/g f.w.	units	units/mg protein
1. IAA 5mM	0.224 ¹	8.409 CDEFG ²	344.107 BCD
2. IAA 5x10 ⁻² mM	0.0197	8.749 BCDEFG	358.247 ABCD
3. IAA 5x10 ⁻⁴ mM	0.0203	8.526 BCDEFG	420.00 ABCD
4. GA ₃ 3mM	0.0196	9.650 ABCDEF	390.686 ABCD
5. GA ₃ 3x10 ⁻⁴ mM	0.0202	7.411 FG	368.798 ABCD
6. Kinetin 5 mM	0.0247	8.150 DEFG	331.262 D
7. Kinetin 5x10 ⁻⁴ mM	0.0205	7.908 EFG	346.947 BCD
8. ABA 2 mM	0.0187	9.685 ABCDE	418.436 ABCD
9. ABA 2x10 ⁻⁴ mM	0.0207	10.105 ABCDE	440.838 ABCD
10. IAA+GA ₃	0.0203	8.784 BCDEFG	379.893 ABCD
11. IAA+Kinetin	0.0213	10.383 ABC	445.515 AB
12. IAA+ABA	0.0189	8.445 CDEFG	446.825 AB
13. IAA+Ethrel	0.0235	10.672 AB	458.613 A
14. GA ₃ +Kinetin	0.0209	9.602 ABCDEF	397.665 ABCD
15. GA ₃ +ABA	0.0204	6.823 G	334.460 CD
16. GA ₃ +Ethrel	0.0274	7.972 EFG	339.409 CD
17. Kinetin+ABA	0.0242	9.577 ABCDEF	420.845 ABCD
18. Kinetin+Ethrel	0.0226	8.686 BCDEFG	386.977 ABCD
19. ABA+Ethrel	0.0226	8.686 BCDEFG	386.977 ABCD
20. Ethrel 500 ppm	0.0264	10.664 AB	434.630 ABCD
21. C-H 2.5 mg/l	--	--	--
22. Act.-D	0.0140	3.566 H	392.350 ABCD
23. Control (water)	0.0296	10.229 ABCD	456.760 AB
LSD	non-significance	2.20	107.506

¹ Average of 3 replications with 2 fruits per sample.

² Means with the same letter are not significantly different at K=100 (± 5% probability level)

Data was tested by Waller-Duncan k ratio t-test.

Table 14. Protein content and peroxidase activity of 'Floradel' tomato at pink stage, when treated with plant growth regulators and actinomycin D at mature-green stage, then held at 21° C

Treatments	Protein content	Peroxidase	
		Total activity	Specific activity
	mg/g f.w.	units	units/mg protein
1. IAA 5mM	0.0265 ¹ AB ²	6.775 BCD	256.676
2. IAA 5x10 ⁻²	0.0247 AB	7.016 ABCD	286.866
3. IAA 5x10 ⁻⁴	0.0223 AB	7.640 ABCD	352.550
4. GA ₃ 3mM	0.0274 AB	7.370 ABCD	267.680
5. GA ₃ 3x10 ⁻⁴ mM	0.0212 BC	6.913 BCD	295.501
6. Kinetin 5 mM	0.0265 AB	7.705 ABCD	296.842
7. Kinetin 5x10 ⁻⁴ mM	0.0253 AB	7.430 ABCD	291.925
8. ABA 2 mM	0.0258 AB	7.300 ABCD	287.435
9. ABA 2x10 ⁻⁴ mM	0.0256 AB	8.415 ABC	341.951
10. IAA+GA ₃	0.0278 AB	6.569 BCD	243.676
11. IAA+Kinetin	0.0298 A	6.506 BCD	222.257
12. IAA+ABA	0.0239 ABC	6.278 CD	262.546
13. IAA+Ethrel	0.0261 ABC	7.523 ABCD	290.672
14. GA ₃ +Kinetin	0.0270 ABC	7.362 ABCD	271.909
15. GA ₃ +ABA	0.0215 BC	7.858 ABC	402.306
16. GA ₃ +Ethrel	0.0241 ABC	7.687 ABCD	319.031
17. Kinetin+ABA	0.0253 AB	7.122 ABCD	290.513
18. Kinetin+Ethrel	0.0246 AB	8.959 ABC	338.681
19. ABA+Ethrel	0.0237 AB	9.597 A	405.525
20. Ethrel 500 ppm	0.0257 AB	9.133 AB	356.589
21. C-H 2.5 mg/l	--	--	--
22. Act.-D	0.0167 C	5.135 D	307.786
23. Control (water)	0.0273 AB	7.360 ABCD	269.644
LSD	0.00765	2.672	non-significance

¹ Average of 3 replications with 2 fruits per sample.

² Means with the same letter are not significantly different at K=100 (± 5% probability level).

Data was tested by Waller-Duncan k ratio t-test.

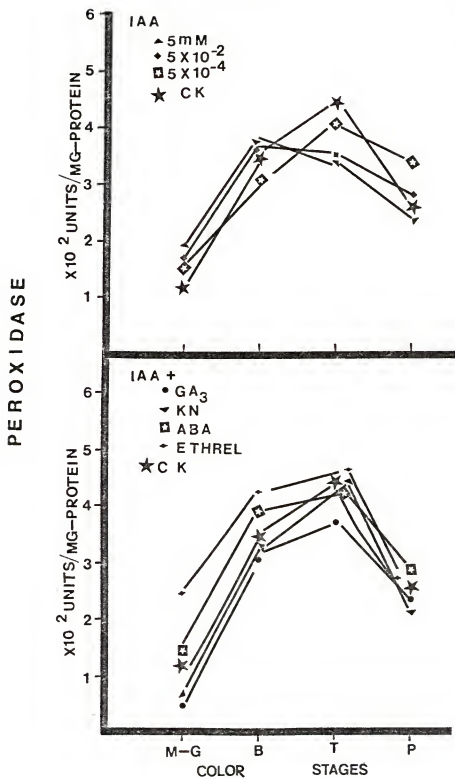


Figure 21. Influence of IAA treatments on peroxidase specific activity of 'Floradel' tomato at designated stages of color development.

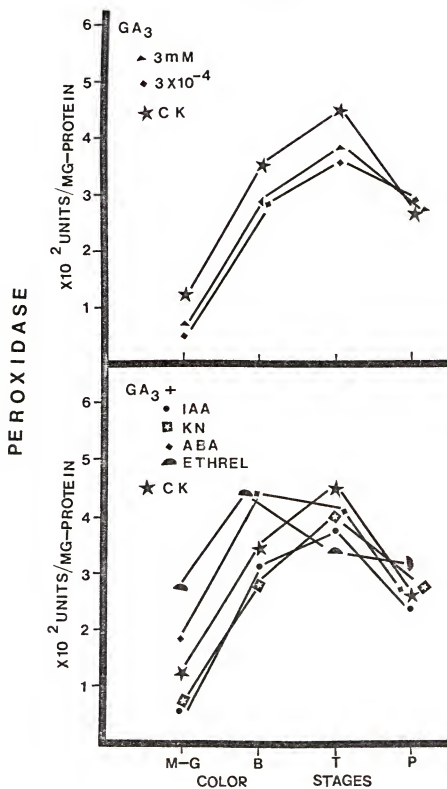


Figure 22. Influence of gibberellin A₃ on peroxidase specific activity of 'Floradel' tomato at designated stages of color development.

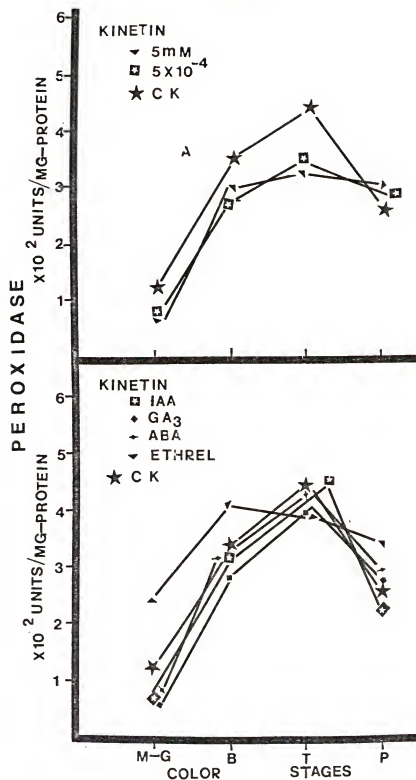


Figure 23. Influence of Kinetin on peroxidase specific activity of 'Floradel' tomato at designated stages of color development.

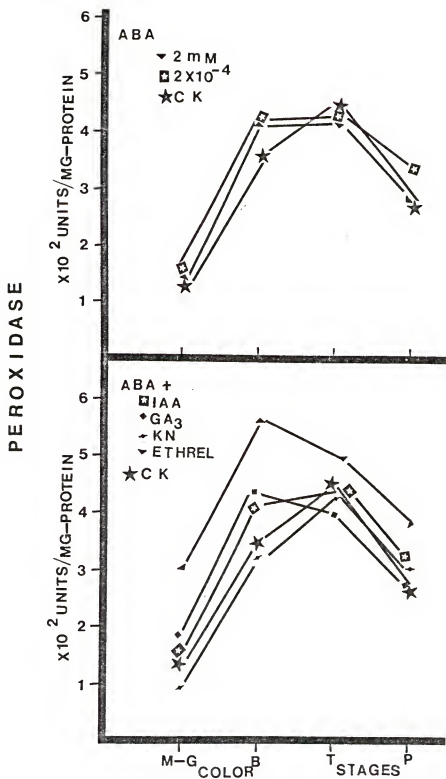


Figure 24. Influence of abscisic acid treatments on peroxidase specific activity of 'Floradel' tomato at designated stages of color development.

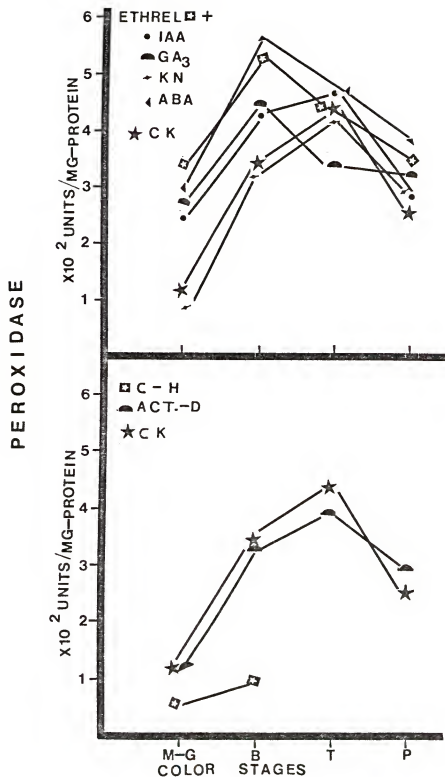


Figure 25. Influence of protein synthesis inhibitors and Ethrel treatments on peroxidase specific activity of 'Floradel' tomato at designated stages of color development.

effects in the combination of Kinetin+GA₃. However, there was an antagonistic effect when fruit were treated with Kinetin+ABA, but the resulting difference was not significant as compared with the control (Fig. 22).

Fruits treated with ABA had a higher peroxidase activity up to the breaker stage at which time activity decreased and then remained constant until the turning stage. Combinations of ABA+IAA and ABA+Kinetin resulted in an intermediate activity level and attainment of the peak was delayed until the turning stage. During the mature-green and breaker stage, ABA+Kinetin actually reduced activity as compared to the control (Fig. 23).

Ethrel also caused a rapid increase in the specific activity and the peak as reached at breaker stage. A slightly synergistic effect occurred when treated with Ethrel+ABA. Kinetin and GA₃ combined with Ethrel delayed attainment of the peak of activity (Fig. 24).

The protein synthesis inhibitor, actinomycin-D, substantially lowered the protein content and enzyme activity at each color stage (Fig. 22).

Electrophoresis and Isoenzyme Pattern (Zymogram)

Comparisons of 'Floradel' and 'Rin' Tomato at Designated Stages of Color Development

Tomato isoenzymes separated by electrophoresis appeared as a brown color in peroxidase when detected with o-dianisidine-H₂O₂ and as a violet color in IAA oxidase when detected with DMACA (p-N, N-Dimethylaminocinnamaldehyde). Both staining procedures detected the same enzyme. A total of nine isoenzymes were detected in 'Floradel' tomato and were coded from a to i. Their rate of mobility ($\pm R_f$) value was 0.016, 0.05,

0.29, 0.49, 0.54, 0.63, 0.68, 0.74, and 0.79, respectively, starting from anode to cathode. These isoenzymes were all anionic; cationic isoenzyme was non-detectable (Fig. 27). Comparisons of isoenzyme pattern with 'Floradel' and 'Rin' tomato at designated stages of color development are shown in Fig. 27, 28.

The zymogram of 'Floradel' at the mature-green stage did not contain isoenzymes b and c. The former (b) appeared when fruit attained the turning stage, the latter (c) appeared when fruit reached the breaker stage. Isoenzyme f possessed strong color intensity and a wider band than e isoenzyme at the mature-green and breaker stages, whereas e isoenzyme was more intense than f isoenzyme at the turning and pink stage (Fig. 28).

Zymograms of 'Rin' tomato did not contain b and i isoenzymes compared with 'Floradel' tomato and there were no changes in isoenzyme pattern among treatments during fruit color development. Strong color intensity developed in h and f isoenzyme in all color stages of 'Rin' fruit (Fig. 28).

Effect of Plant Growth Regulators on the Isoenzyme Pattern of 'Floradel' Tomato at Designated Stages of Color Development

Mature-green (M-G) stage. Fruits treated with IAA (5 mM - 5×10^{-4} mM) alone hastened the appearance of c isoenzyme (Fig. 25); however, a isoenzyme was inhibited by Kinetin and ABA treatments (Fig. 30, 31). Combinations of IAA+Kinetin, IAA+ABA and Kinetin+ABA also showed the inhibition of a isoenzyme (Fig. 25, 26).

Generally, IAA, GA_3 , and Kinetin treatments decreased one or several isoenzyme activities in terms of color intensity, whereas ABA and Ethrel increased the activity. Combinations of IAA, Kinetin, and GA_3 also showed a decreasing effect on some enzyme activities (Fig. 29



Figure 26. Isoenzyme pattern of 'Rin' tomato at mature-green, green-yellow, yellow-green, and yellow color stages (from left to right) compared with 'Floradel' tomato at breaker stage (right).

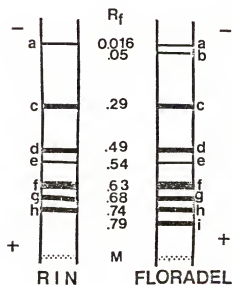


Figure 27. Mobility rate ($\pm R_f$) of anionic peroxidase isoenzymes of 'Rin' and 'Floradel' tomatoes after electrophoresis on polyacrylamide gels.

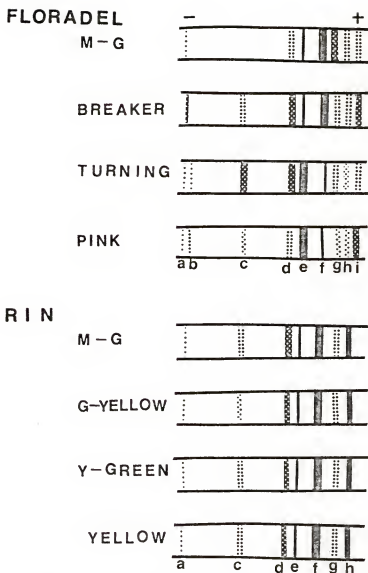


Figure 28. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' and 'Rin' tomato at designated stages of color development.

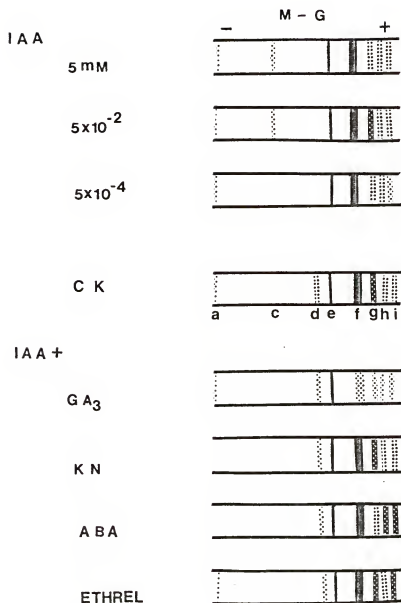


Figure 29. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with IAA treatments at mature-green stage.

30, 32); ABA+GA₃ and ABA+Kinetin decreased some enzyme activity, but ABA+IAA and ABA+Ethrel increased the activity compared to the control (Fig. 31). Ethrel and Ethrel+ABA increased all the isoenzymes except a isoenzyme. Ethrel+IAA increased activity of i isoenzyme while Ethrel+Kinetin and Ethrel+GA₃ increased activity of d isoenzyme. Protein inhibitors, cycloheximide and actinomycin D did not change the isoenzyme pattern; however, activity was sharply decreased in most isoenzymes (Fig. 33).

Breaker stage. Isoenzymes increased their activity in this stage. Adequate amount of protein for separating isoenzyme was 15 µg per gel instead of 25 µg at the mature-green stage.

Tomatoes treated with IAA, GA₃, and Kinetin, alone or in combinations, had a decreased activity in one or several isoenzymes in terms of color intensity compared to the control (Fig. 34-36). Treatment with ABA and Ethrel, alone or in combination, showed increasing activity (Fig. 37-38). Kinetin, GA₃, and IAA combined with ABA or Ethrel also increased the isoenzyme activity as compared with the control (Fig. 37-38). Cycloheximide treatment decreased almost all isoenzyme activity whereas actinomycin D affected only the a isoenzyme (Fig. 38).

Turning stage. A new isoenzyme b appeared in this color stage and e isoenzyme band become more intense as compared to band size in mature-green and breaker stages. There were no differences in isoenzyme patterns due to treatment.

Fruits treated with IAA, GA₃, and Kinetin alone or in combination decreased their isoenzyme activity (Fig. 39-41). Fruits treated with ABA or Ethrel per se did not exhibit a significant increase in activity, but where GA₃ was used in the combination there was a decreased

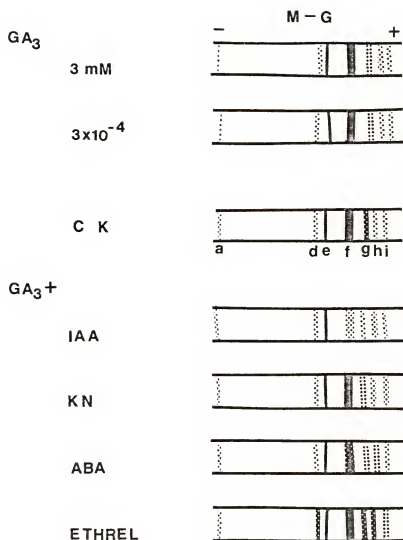


Figure 30. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with gibberellin A₃ treatments at mature-green stage.

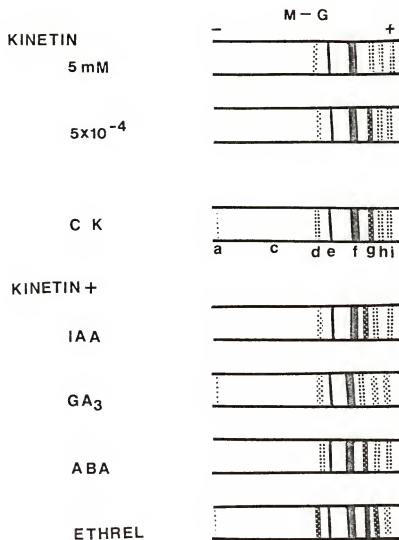


Figure 31. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with Kinetin treatments at mature-green stage.

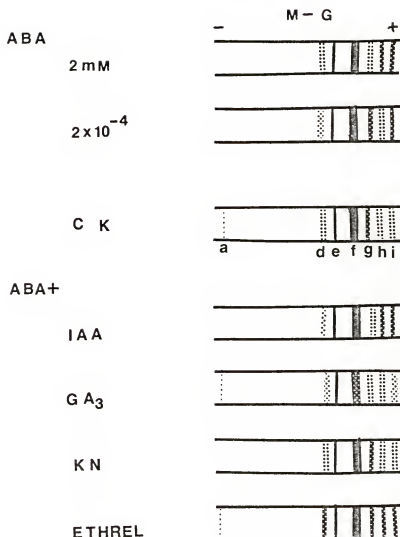


Figure 32. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with abscisic acid treatments at mature-green stage.

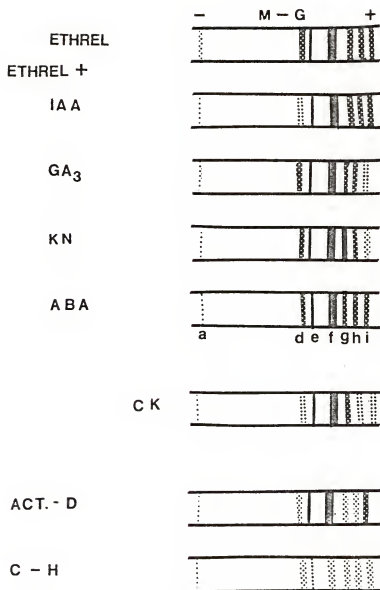


Figure 33. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with protein synthesis inhibitors and Ethrel treatments at mature-green stage.

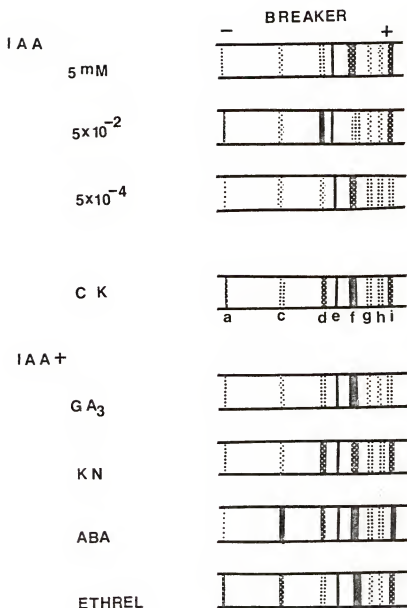


Figure 34. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with IAA treatments at breaker stage.

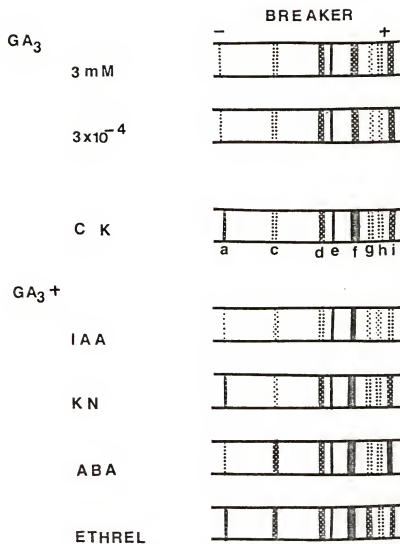


Figure 35. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with gibberellin A₃ treatments at breaker stage.

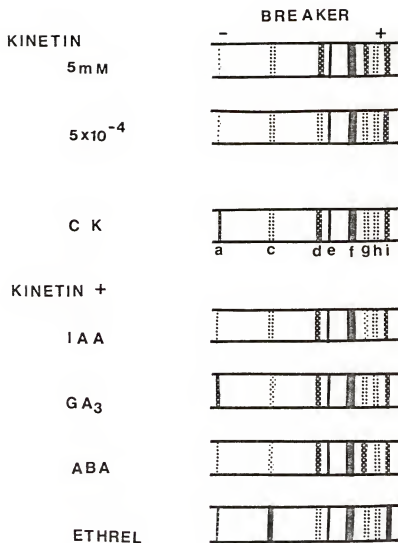


Figure 36. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with Kinetin treatments at breaker stage.

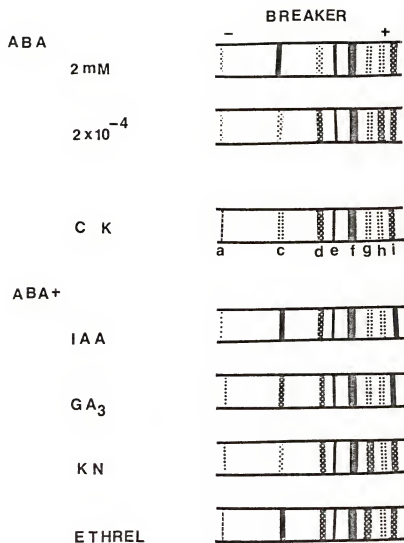


Figure 37. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with abscisic acid at breaker stage.

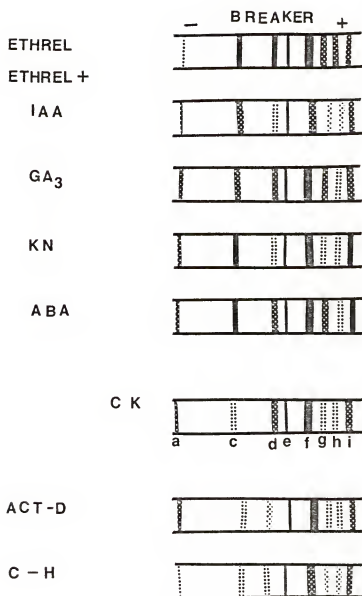


Figure 38. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with protein synthesis inhibitors and Ethrel treatments at breaker stage.

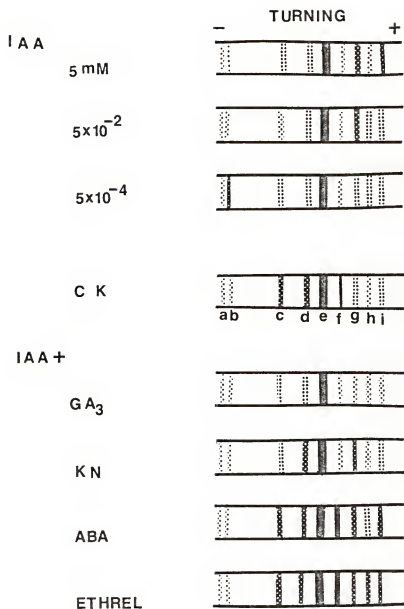
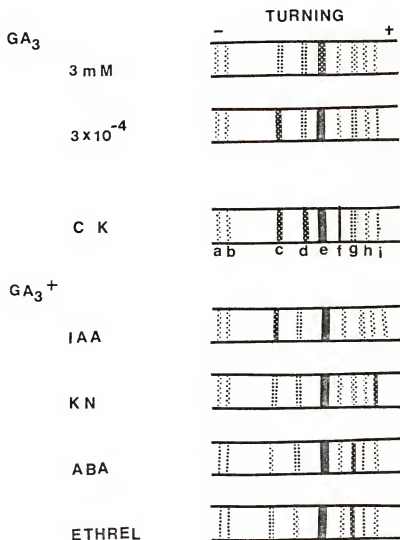


Figure 39. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with IAA treatments at turning stage.



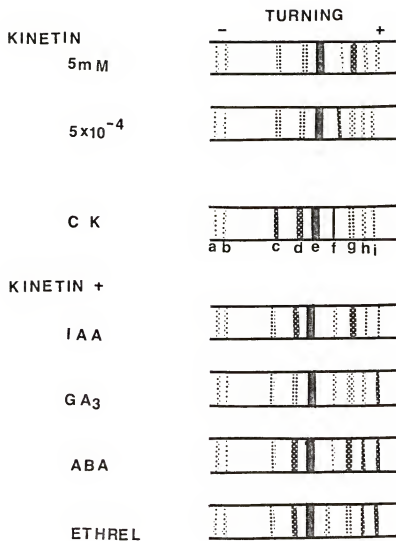


Figure 41. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with Kinetin treatments at turning stage.

activity (Fig. 42, 43), Combination of ABA+Ethrel showed a strongly increased activity, Actinomycin D caused a reduced isoenzyme activity in this color stage (Fig. 43).

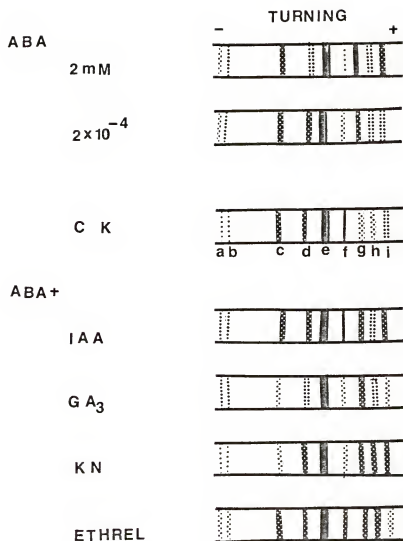


Figure 42. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with abscisic acid treatments at turning stage.

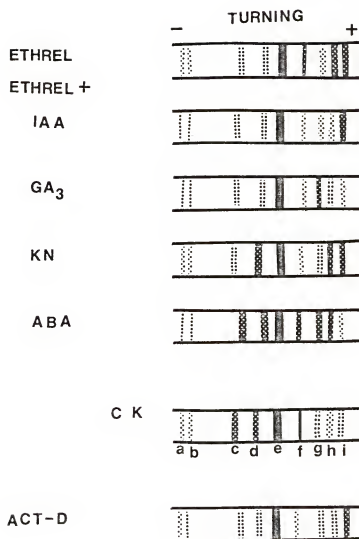


Figure 43. Isoenzyme pattern of peroxidase on polyacrylamide gel from 'Floradel' tomato with actinomycin D and Ethrel treatments at turning stage.

DISCUSSION

Methodology

Solution infiltration with vacuum has been found satisfactory in this experiment. A testing solution with a blue dye was evenly penetrated into placental tissue through vascular bundles when the vacuum returned to atmospheric pressure (Fig. 44). This method also gave good results in pome fruits (54). However, the operation should involve slow vacuum return to atmospheric pressure since tissue is easily damaged when the solution enters into the fruit rapidly and in large amounts.

Polyvinylpyrrolidone (PVP), a tanning and phenol scavenger, has been successfully applied in many enzyme studies (91, 92, 140), but was not satisfactory in this tomato study without modification. Further partial purification with ammonium sulfate fractionation and dialysis was needed to get a higher activity. This was also the case in liverwort extracts (152).

Respiration, Ethylene Production and Color Development

The essential elements in the fruit-ripening complex might be considered to be a respiratory source of energy for ripening, for the synthesis or formation of a new set of enzymes, and the actions of these enzymes to bring about the changes characteristic of ripening such as softening, pigmentation, and quality changes in the fruits. A supposition is made that a small amount of energy is enough for formation of those ripening enzymes. This might be explained in that non-climacteric fruit can ripen without upsurged respiration and non-phosphorylation, cyanide-

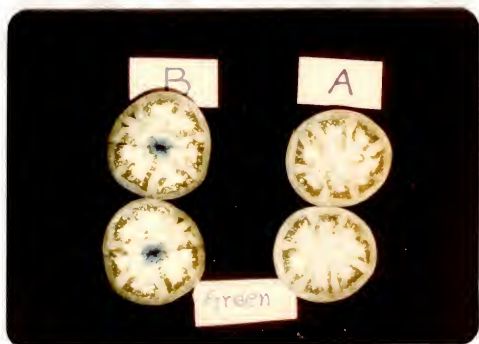


Figure 44. Blue dye penetration into fruit through vascular bundles by vacuuum infiltration (A) and syringe injection (B).

insensitive respiratory pathway is coupled with fruit ripening in climacteric fruits,

The ability of a fruit to become ripe is closely related to some changes experienced at maturity. Massive doses of ethylene can bring about ripening changes in immature tomato fruit, but as a fruit reaches maturity, its response to the ethylene trigger is enormously increased. It has been suggested that some factor (hormone) supplied by the tree defers ripening (12).

Plant hormone either endogenous or exogenously applied can serve to regulate fruit ripening (41). The potential of auxin, gibberellin and benzyladenine to counteract the effects of Ethrel and abscisic acid on tomato fruit ripening has been examined over a wide range. Indoleacetic acid, GA_3 , and BA opposed the ethylene and abscisic acid in respiration, ethylene evolution, and color development. However, they did not change the normal pattern of ripening in the tomato fruit. Supplementary GA_3 and BA caused delay in ripening of tomato fruit and accounts for decreasing the activity of peroxidase, thence, decreasing ethylene formation. It was found that IAA increased the peroxidase activity of tomato fruits but inhibited tomato fruit ripening. This might be explained in that IAA stimulated the action of "Juvenility factors" which increased the threshold level where ethylene initiates tomato ripening (51). Gibberellin delayed ripening of tomato not only in slow color development but also in delaying the onset of climacteric and prolonging the time to attain the peak both in respiration and ethylene production. These results are in agreement with some (9) but contrary to others (43).

Other data (83) have indicated that ABA is as effective as ethylene or Ethrel in tomato ripening. However, in data presented here Ethrel

was shown to be more effective in tomato ripening than ABA treatment either at 2 mM or at 2×10^{-4} mM. Color development as a result of treatment with ABA was about 2 days slower as compared to Ethrel treatment. The stimulation by Ethrel was most apparent during the first six days of ripening. This supports the results obtained by Gull (68). It is suggested that ABA restricted color development by decreasing the internal ethylene. This was demonstrated in ethylene evolution results where 2 additional days were required to attain the peak in ABA treatment as compared to Ethrel treatment. Although ABA increases ethylene production in carnation and in fruit (32, 111), it is not as effective as Ethrel. It is also possible that ABA and Ethrel act at different sites inside the fruit since the hypothesis is that ABA increases lycopene synthesis and ethylene increases chlorophyll degradation and then increases lycopene (86).

Antagonism effects were found in the results when GA_3 or Kinetin were combined with ABA or Ethrel. When GA_3 and Ethrel were combined, ripening of tomato was restored to a normal rate. This is similar to the results obtained when gibberellin was applied before ethylene (43). The climacteric pattern in this combination was close to treatment with Ethrel alone. This singular insensitivity of the respiratory climacteric to gibberellin indicates that the ripening processes (color developments) are not all closely linked with the respiratory shifts associated with the climacteric (30). The climacteric pattern was shifted in Kinetin+Ethrel combination where the time required to attain the peak was increased. This can be explained by the fact that Kinetin and Ethrel work at the protein synthesis enzyme level. A scheme of allosteric effects has been proposed and demonstrated with invertase activity in

that ABA or ethylene act as a negative allosteric effector (inhibitor) on biologically active proteins; the positive effector (or activator) may be GA_3 , Kinetin or IAA (137).

Protein inhibitors, actinomycin D and cycloheximide, have showed substantial effects on tomato ripening in these results. A tremendously high respiration rate and slightly high ethylene production was found in fruits treated with cycloheximide. This is suggestive that cycloheximide completely inhibited or is toxic to metabolism, weakens tissue and allows contamination by organisms. The breakdown symptom is very close to that developed in anaerobic condition. It is possible that cycloheximide inhibits the formation of pyruvate dehydrogenase system or other enzymes in tricarboxylic cycle (Kreb cycle) resulting in anaerobic respiration which provides no energy for metabolism and ethylene evolution, but a normal climacteric during the experiment. Color developed slowly and normally. However, some fruits showed an oily light blotch in the skin. It is suggested that actinomycin D probably affects cellular metabolism other than inhibition of protein synthesis.

It is noteworthy that 'rin' tomato, treated with plant growth regulator, had no stimulation of ethylene production and respiration except a temporary increased respiration by Ethrel. These results are similar to others (78, 120). Loss of ripening ability in 'Rin' tomato could be due to a lack of a gene or gene regulator to receive the ripening-onset signal from "the balance of hormone pool" followed by no ripening enzyme activation or formation.

Protein Synthesis and Peroxidase Activity

Increased protein synthesis during the climacteric, reported in avocado and tomato (9, 131), was not observed in this study. Results in this study may be due to the inherent low protein content in tomato and to the further reduction that took place during partial purification of enzyme with ammonium sulfate. However, protein inhibitors, cycloheximide and actinomycin D, caused significantly lower protein content during the experiment.

In these results, IAA oxidase and peroxidase, activities expressed either in total activity or specific activity, were similar at corresponding fractionation levels. Furthermore, electrophoresis on acrylamide gels gave the same number of isoenzymes with identical mobilities. This indicated that IAA oxidase activity is related to oxidase function of peroxidase and is consistent with other reports (146, 155) although a specific non-peroxidative IAA oxidase has been reported (142).

Results showed that peroxidase sharply increased its specific activity about 3 fold as fruit color developed from mature-green to breaker stage. This is in agreement with other observations (95). Generally, the climacteric is initiated before breaker stage and attained its peak at the light pink stage (120, 174).

A promotive effect on peroxidase activity was found in this study when tomato fruit were treated with IAA, and this is similar to the result obtained with etiolated pea tissue (59). Since activity was increased in proportion to IAA concentration and had no "dual effect" shown in concentration, IAA is speculated as a substrate activator like glucuronides in β -Glucuronidase (47). It is suggested that additional studies be initiated since low IAA content in tomato has been analyzed in the mature-green stage (45).

Results obtained from GA_3 treatments showed inhibition of peroxidase activity. Such inhibition has been reported previously and hypothesized to be due to GA_3 reducing the destruction of endogenous IAA (20). Other supporting data show that dwarf maize seedling have abnormally high peroxidase activity, but when they are treated with gibberellin to relieve dwarfism, their peroxidase activity falls precipitously (113). Kinetin inhibited peroxidase activity has also been shown in tobacco callus cells (100).

Ethylene resulted in an increase in peroxidase activity in this study. Ethylene has also been reported to increase the activity of peroxidase in cotton leaves and sweet potato (56, 79, 156).

The onset of the climacteric and color development coincided with enzyme activity, except for the IAA treatment. This is in agreement that peroxidase is related to the ethylene synthesis (94). Thus, high peroxidase activity could increase ethylene in fruit and attain the threshold level to induce the climacteric rise and initiate color development. Fruit treated with IAA may not only increase the internal ethylene but also stimulate the "juvenility factors" in desensitizing fruit to exogenous or endogenous ethylene (49). Indoleacetic acid increased peroxidase activity without inducing ripening, perhaps for the same reason.

It is hypothesized that H_2O_2 is involved in oxidative processes required in the initiation and the promotion of ripening (19). Peroxidase is a peroxide utilizing enzyme (8). It might control fruit ripening by regulating the H_2O_2 utilization. It might also be involved in the oxidative breakdown of IAA resulting in a decrease in "juvenility

factors" and formation of IAA oxidation product which can promote senescence in fruits (52).

'Rin' tomato had no change in peroxidase activity during color development. One of the reasons could be that no change in ethylene evolution occurred during the experiment. Free methionine is considered to be a primary precursor of ethylene and it occurs in similar levels in both 'rin' and normal tomato (64). Thus, the precursor is certainly not limiting. Therefore, inactivity of peroxidase and ethylene evolution are consistent.

Isoenzymes vs. Plant Growth Regulators and Protein Inhibitors

Isoperoxidase of horse-radish has been classified into cationic and anionic isoenzymes (84, 144). Our results showed that no cationic isoenzyme was separated in any color stage. This supports other observations on tomato (95).

Results demonstrated that plant hormone and successive ripening stages can induce qualitative as well as quantitative changes in the isoperoxidases of tomato fruits. This should remove the doubts about the reality of the phenomenon and permit us to consider its nature and possible regulatory significance (16). We have found isoenzyme c which is normally absent in mature-green fruit but present in other color stages. This isoenzyme was identified in mature-green fruit in conjunction with IAA. Indoleacetic acid may induce some and repress other peroxidase isoenzymes (57). It is also reported that certain peroxidase isoenzymes are more active than others in IAA destruction. Thus, it would appear that IAA may regulate the extent of its own destruction by altering the level of the various peroxidase isoenzymes produced

(143). It is speculated that isoenzyme c may play a significant role in enzyme activity and fruit ripening since it coincides with enzyme activity change and color development.

Of interest is the relation found between the appearance of isoenzyme f which increased in activity and amount (expressed as color density and width of band on electrophoresis gel) at mature-green and breaker stage. On the contrary, isoenzyme e instead of isoenzyme f increased at the turning and pink stages. It is suggested that those changes act as an indication of the postclimacteric period.

'Rin' tomato did not change its isoenzyme pattern during color development. However, the rapidly mobile isoenzyme i and isoenzyme b were lost compared to the 'Floradel' tomato. Possibly it is due to a different genetic type than 'Floradel' since different isoenzyme patterns appeared in different varieties (144).

Different isoperoxidases have different relative affinities toward a variety of substrate, the appearance of new isoenzyme could be a signal of new biochemical capacities within the tissue (107, 112). Since the activity of isoenzyme i expressed coincidence with fruit ripening in 'Floradel' tomato, the gene or gene system controlling this isoenzyme formation might be considered as an important role in inhibiting 'Rin' fruit ripening.

The fact that new isoemzymes appear at various ripen stages with no sensitivity of both induction and repression to cycloheximide and actinomycin D indicated that their appearance is not due to de novo synthesis. This result suggested that m-RNAs for peroxidase synthesis preexists in the cell.

From the studies above, data support the theory that plant growth regulators applied exogenously will directly affect tomato fruit ripening by mediating peroxidase activity and their isoenzyme pattern and consequently regulate the production of ethylene which then induces ripening.

CONCLUSION AND SUMMARY

The ripening changes of tomatoes (cv. 'Floradel') treated with plant growth regulators appeared to follow a predetermined pattern. Gibberellin A_3 , Kinetin, and IAA, in decreasing order of effectiveness, inhibited ripening of tomato when treated alone or in combination, whereas ABA and Ethrel hastened ripening.

Antagonistic effects on respiration and ethylene evolution resulted from the combination treatments of GA_3 or Kinetin plus ABA or Ethrel, whereas synergistic effects were found in the GA_3 +Kinetin treatment.

Peroxidase activity in control treatment increased about 3 fold as fruit ripened from mature-green to the turning stage. Gibberellin A_3 and Kinetin decreased enzyme activity, whereas IAA, ABA, and Ethrel increased activity in both mature-green and breaker stage. Increase or decrease of enzyme activity was directly proportional to the onset of the climacteric and color development.

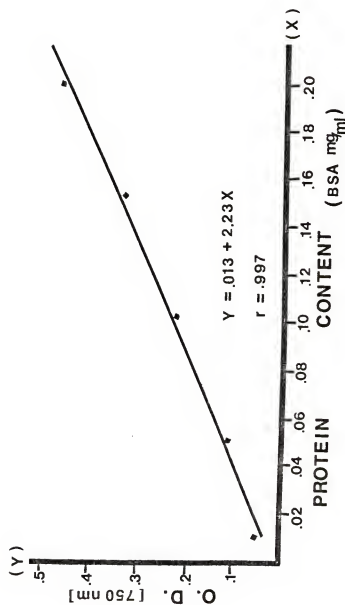
The isoenzyme pattern of peroxidase can be influenced by the plant growth regulators. An isoenzyme c which is affected by IAA in high concentration ($5 - 5 \times 10^{-2}$ mM) has appeared when fruit attained the breaker stage. A rapidly mobile isoenzyme i and a slowly mobile isoenzyme b did not exist in 'Rin' variety when compared to 'Floradel' tomatoes. It is suggested that isoenzyme c relate to fruit ripening and isoenzyme i relate to the inhibition of ripening in 'Rin' tomatoes.

'Rin' tomatoes treated with growth regulator had no ethylene evolution or respiration stimulation except a temporary rise in respiration with the Ethrel treatment. Growth regulator did not affect the peroxidase activity, and may be the reason why ethylene remained unchanged.

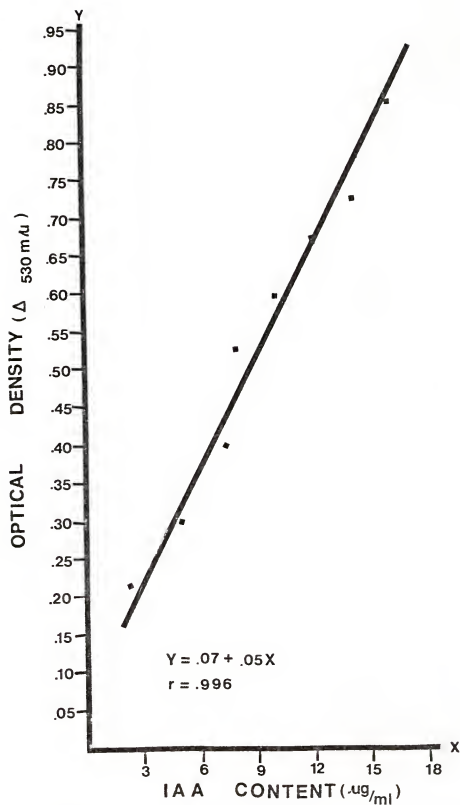
Protein inhibitors decreased protein content in both 'Floradel' and 'Rin' tomatoes. Cycloheximide inhibited the peroxidase activity but actinomycin D did not. The results suggested that m-RNA for peroxidase synthesis preexists in the cell.

A hypothesis is proposed that fruit ripening is caused by changes in hormone pool sizes inside the tissue. A ripening-induced signal is generated when the composition of each constituent attains a proper ratio which can be modulated by different environments such as temperature, light, fertilizer, age, management, and exogenous plant growth regulators. The ethylene-forming essential enzyme(s), such as peroxidase, is thought to be the best candidate to accept this signal to initiate fruit ripening.

A P P E N D I X



Appendix 2. A standard curve for protein measurement by Lowry Method.



Appendix 3. A standard curve for IAA oxidase activity measurement.

BIBLIOGRAPHY

1. Abeles, F. B. 1972. Biosynthesis and mechanism of action of ethylene. *Ann. Rev. Plant Physiol.* 23:259-292.
2. Abdel-Gawad, H. A. and R. J. Romani. 1967. Effects of phytohormones on maturation and postharvest behaviour of fruit. *Plant Physiol. Suppl.* 42:s-43.
3. Abdel-Kadar, A. S., L. L. Morris, and E. C. Maxie. 1966. Effect of growth regulating substance on the ripening and shelf-life of tomatoes. *HortScience* 1(3-4):90-91.
4. Abdel-Rahman, M., T. H. Thomas, G. J. Doss, and L. Howell. 1975. Changes in endogenous plant hormones in cherry tomato fruits during development and maturation. *Physiol. Plantum* 34:39-43.
5. Aharoni, N., A. Back, S. Ben-Yehoshua, and A. E. Richmond. 1975. Exogenous gibberellic acid and the cytokinin isopentenyladenine retardants of senescence in Romaine lettuce. *J. Am. Soc. Hort. Sci.* 100(1):4-6.
6. Akiva, A., E. E. Goldschmidt, and S. Ben-Yehoshua. 1976. Involvement of endogenous ethylene in the induction of color change in 'Shamouti' oranges. *Plant Physiol.* 57:836-838.
7. Anonymous. 1966. *Munsell book of color.* Munsell Color Company, Inc., Baltimore, Maryland. 13 pp.
8. Anonymous. 1972. *The Worthington enzyme manual.* Worthington Biochem. Corp. Freehold, New Jersey. 216 pp.
9. Babbitt, J. K., M. J. Powers, and M. E. Patterson. 1973. Effects of growth regulators on cellulase, polygalacturonase, respiration, color and texture of ripening tomatoes. *J. Am. Soc. Hort. Sci.* 98(1):77-81.
10. Ballantyne, D. J. 1966. Respiration of floral tissue of the daffodil treated with benzyladenine and auxin. *Can. J. Botany* 44:117.
11. Bendall, D. S. and W. D. Bonner. 1971. Cyanide-sensitive respiration in plant mitochondria. *Plant Physiol.* 47:236-245.

12. Biale, J. B. 1960. Respiration of fruits. *Handbuch der Pflanzen Physiologie*, (W. Ruhland, ed.) 12(2):536-591, Springer, Verlag, Berlin.
13. Biale, J. B. 1969. Metabolism at several levels of organization in the fruit of the avocado, *Persea americana* Mill. Qual. Plant. Mater, Veg, 19(1-3):141-153.
14. Blackman, F. F. and P. Pariza. 1928. Analytic studies in plant respiration. 1. The respiration of a population of senescent ripening apples. *Proc. Roy. Soc. Ser. B.* 103:412-415. (cited from Sacher, J. A. and S. Salminen. 1969. *Plant Physiol.* 44: 1371-1377).
15. Blanpied, G. D. and E. Hansen. 1968. The effect of oxygen, carbon dioxide, and ethylene on the ripening of pears at ambient temperatures. *Proc. Am. Soc. Hort. Sci.* 93:813-816.
16. Boll, W. G. 1965. On the claimed 'adaptive' nature of IAA oxidase and an effect of green light on IAA oxidase activity. *Can. J. Bot.* 43:855-892.
17. Brady, C. J., P. B. H. O'Connell, J. Smydzuk, and N. L. Wade. 1970. Permeability, sugar accumulation, and respiration rate in ripening banana fruits. *Aust. J. Biol.* 23:1143-1152.
18. Brady, C. J., J. K. Palmer, P. B. H. O'Connell, and R. M. Smillie. 1970. An increase in protein synthesis during ripening of the banana fruit. *Phytochemistry* 9:1037-1047.
19. Brennan, T. and C. Frenkel. 1977. Involvement of hydrogen peroxide in the regulation of senescence in pear. *Plant Physiol.* 59:411-416.
20. Brian, P. W. 1966. The gibberellins as hormones. *Int. Rev. Cyto.* 19:229-259.
21. Brisker, H. E., E. E. Goldschmidt, and R. Goren. 1976. Ethylene-induced formation of ABA in citrus peel as related to chloroplast transformations. *Plant Physiol.* 58:377-379.
22. Burg, S. P. 1962. The physiology of ethylene formation. *Ann. Rev. Plant Physiol.* 13:265-320.
23. Burg, S. P. and E. A. Burg. 1965. Ethylene action and the ripening of fruit. *Science* 148:1190-1196.
24. Burg, S. P. and E. A. Burg. 1965. Relationship between ethylene production and ripening in banana. *Bot. Gaz.* 126:200-204.

25. Burg, S. P. and E. A. Burg. 1966. Fruit storage at subatmospheric pressures. *Science* 153:314-315.
26. Burg, S. P. and E. A. Burg. 1969. Interaction of ethylene oxygen and carbon dioxide in control of fruit ripening. *Qual. Plant. Mater. Veg.* 19:185-200.
27. Cline, M. G. 1976. Some common misconceptions concerning hormone experiments with excised plant tissue. *Plant Science Letter* 6:181-184.
28. Coggins, Jr., C. W. and L. W. Lewis. 1962. Regreen of valencia orange as influenced by potassium gibberellin. *Plant Physiol.* 37:625-627.
29. Colquhoun, A. J. and J. R. Hillman. 1975. Endogenous abscisic acid and the senescence of leaves of Phaseolus vulgaris L. *Z. Pflanzen* 76:326-332.
30. Coombe, B. G. 1976. The development of fleshy fruits. *Ann. Rev. Plant Physiol.* 27:207-228.
31. Cooper, W. C. and W. H. Henry. 1971. Abscission chemicals in relation of citrus fruits harvest, *J. Agr. Food Chem.* 19: 559.
32. Cracker, L. E. and F. B. Abeles. 1969. Abscission: Role of abscisic acid. *Plant Physiol.* 44:1144-1149.
33. Crane, J. C. 1964. Growth substance in fruit setting and development. *Ann. Rev. Plant Physiol.* 17:459-480.
34. Crispeels, M. J. and J. E. Varner. 1967. Hormonal control of enzyme synthesis: on the mode of action of GA and Abscisin in aleurone layers of Barley. *Plant Physiol.* 42:1008-1016.
35. Crocker, W., P. W. Zimmerman, and A. E. Hitchcock. 1935. Gas injury to rose in green house. *Contr. Boyce Thompson Inst. Plant. Res.* 7:231-248.
36. Davis, B. J. 1964. Disc electrophoresis II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* 121:404-427.
37. De Leo, P. and J. A. Sacher. 1970. Control of ribonuclease and acid phosphatase by auxin and ABA during senescence of Rhoeo leaf section. *Plant Physiol.* 46:806-811.
38. De Leo, P. and J. A. Sacher. 1971. Effect of ABA and auxin on ribonuclease during aging of bean endocarp tissue sections. *Plant Cell Physiol.* 12:791-796.

39. Dedolph, R. R. and S. Goto. 1960. The ripening of bananas to some growth regulator treatment. *Bot. Gaz.* 121:151-154.
40. Dedolph, R. R., S. H. Wittwer, V. Tuli, and D. Gilbert. 1962. Effect of N^6 -benzylaminopurine on respiration and storage behavior of broccoli (*Brassica oleracea* var. 'italica'). *Plant Physiol.* 37:509-512.
41. Dilley, D. R. 1969. Hormonal control of fruit ripening. *Hort-Science* 4:111-114.
42. Dizengremel, P. and C. Lance. 1976. Control of changes in mitochondrial activities during aging of potato slices. *Plant Physiol.* 58:147-150.
43. Dostal, H. C. and A. C. Leopold. 1967. Gibberellin delays ripening of tomatoes. *Science* 153:314-315.
44. Eilati, S. K., E. E. Goldschmidt, and S. P. Monselise. 1969. Hormonal control of color changes in orange peel. *Experientia* 25:209.
45. El-Beltagy, A. S., J. P. Patrick, E. W. Hervett, and M. A. Hall. 1976. Endogenous plant growth regulator levels in tomato fruits during development. *J. Hort. Sci.* 51:15-30.
46. Fawcett, C. H. 1961. Indole auxin. *Ann. Rev. Plant. Physiol.* 12:239-326.
47. Fishman, W. H. 1955. Beta-Glucuronidase. *Advance Enzymol.* 16:361-391.
48. Frenkel, C. 1972. Involvement of peroxidase and indole-3-acetic acid oxidase isozymes from pear, tomato, and blueberry fruit in ripening. *Plant Physiol.* 49:757-763.
49. Frenkel, C. 1975. Oxidative turnover of auxin in relation to the onset of ripening in 'Bartlett' pears. *Plant Physiol.* 55:480-484.
50. Frenkel, C. and R. Dyck. 1973. Auxin inhibition of ripening in 'Bartlett' pears. *Plant Physiol.* 51:6-9.
51. Frenkel, C., R. Dyck, and N. F. Haard. 1975. Role of auxin in the regulation of fruit ripening. *in: Postharvest Biology and Handling of Fruits and Vegetables.* Chapt. 3, (Haard and Salunkhe, ed.), AVI Publishing, Westport, Connecticut, USA.
52. Frenkel, C., V. R. Haddon, and J. Smallheer. 1975. Promotion of softening and ethylene synthesis in 'Bartlett' pear by 3-methyleneoxindole. *Plant Physiol.* 56:647-649.

53. Frenkel, C. and N. F. Haard. 1973. Initiation of ripening in 'Bartlett' pear with an antiauxin alpha-p-chlorophenoxy isobutyric acid. *Plant Physiol.* 52:380-384.
54. Frenkel, C., I. Klein, and D. R. Dilley. 1968. Protein synthesis in relation to ripening of pome fruits. *Plant Physiol.* 43: 1146-1153.
55. Frenkel, C., I. Klein, and D. R. Dilley. 1969. Methods for the study of ripening and protein synthesis in intact pome fruits. *Phytochemistry* 8:945-955.
56. Gahagan, H. E., R. E. Holm, and F. B. Abeles. 1968. Effect of ethylene on peroxidase activity. *Physiol. Plantum.* 21:1270-1279.
57. Galston, A. W. 1967. Regulatory system in higher plant. *Am. Sci.* 55:147-160.
58. Galston, A. W., J. Bonner, and R. S. Baker. 1950. Flavoprotein and peroxidase: constituents of the indoleacetic acid oxidase of peas. *Am. J. Bot.* 37:677-678.
59. Galston, A. W. and L. Y. Dalberg. 1954. The adaptive formation and physiological significance of indoleacetic acid oxidase. *Am. J. Bot.* 41(5):373-380.
60. Galston, A. W. and P. J. Davies. 1969. Hormonal regulation in higher plant. *Science* 163:1288-1297.
61. Galston, A. W. and W. K. Purves. 1969. The mechanism of action of auxin. *Ann. Rev. Plant Physiol.* 11:239-276.
62. Goldschmit, E. E., S. K. Eilati, and R. Goren. 1972. Increase in ABA-like growth inhibitors and decrease in gibberellin-like substances during ripening and senescence of citrus fruits. *in: Plant Growth Substances*, (D. J. Carr, ed.) 1970, Springer Verlag, Berlin. pp. 611-618.
63. Goldschmidt, E. E. and D. Galiby. 1974. The fate of endogenous gibberellins and applied radioactive gibberellin A_3 during natural and ethylene-induced senescence in citrus peel. *Plant and Cell Physiol.* 15(3):485-492.
64. Gonzalez, A., P. E. Brecht, and C. C. Rehkugler. 1976. Free methionine levels in 'rin' and normal isogenic tomato fruits ripened in the field or in storage. *Plant Physiol.* 58:648-650.
65. Gordon, S. A. and R. P. Weber. 1951. Colorimetric estimation of indoleacetic acid. *Plant Physiol.* 26:192-195.

66. Gorin, N. and F. T. Heidema. 1976. Peroxidase activity in Golden Delicious apples as a possible parameter of ripening and senescence. *J. Agri. Food Chem.* 24(1):200-201.
67. Gove, J. P. and M. C. Hoyle. 1975. The isozymic similarity of indoleacetic acid oxidase to peroxidase in birch and horse-radish. *Plant Physiol.* 56:586-687.
68. Gull, D. D. 1968. Ripening behavior and edible quality of tomato treated with Amchem 68-62 (Ethrel). *Proc. Fla. Stat. Hort. Soc.* 81:215-220.
69. Gull, D. D. 1972. Suppression of ethylene-induced ripening of tomatoes by carbon dioxide. *Proc. Fla. Stat. Hort. Soc.* 85:216-219.
70. Haard, N. F. 1973. Upsurge of particulate peroxidase in ripening banana fruit. *Phytochemistry* 12:555-560.
71. Haard, N. F. and C. L. Tobin. 1971. Patterns of soluble peroxidase in ripening banana fruit. *J. of Food Sci.* 36:854-857.
72. Hale, C. R., B. G. Coombe, and J. S. Hawker. 1970. Effect of ethylene and 2-chloroethylphosphoric acid on ripening of grapes. *Plant Physiol.* 45:620-623.
73. Halevy, A. H. 1963. Interaction of growth-retarding compound and gibberellin on indoleacetic acid oxidase and peroxidase of cucumber seedling. *Plant Physiol.* 38:731-737.
74. Hansen, E. 1946. Effect of 2,4-dichlorophenoxyacetic acid on the ripening of 'Bartlett' pears. *Plant Physiol.* 21:585-592.
75. Hansen, E. 1966. Post-harvest physiology of fruit. *Ann. Rev. Plant Physiol.* 17:459-480.
76. Harkett, P. J., A. C. Hulme, M. J. C. Rhodes, and L. S. C. Woollorton. 1971. The threshold value for physiological action of ethylene on apple fruits. *J. Food Technol. (UK)* 6:39-85.
77. Hartmann, C. 1959. Effect of growth-regulating substances on the carbon dioxide evolution and postharvest ripening of tomatoes. *Plant Physiol.* 34:65-72.
78. Herner, R. C. and K. C. Sink, Jr. 1973. Ethylene production and respiration behavior of the 'rin' tomato mutant. *Plant Physiol.* 52:38-42.
79. Herreo, R. A. and W. C. Hall. 1960. General effects of ethylene on enzyme systems in the cotton leaf. *Plant Physiol.* 13:736-750.

80. Ivanova, J. and A. Ivanova. 1968. The oxidation of grape must III. Polyphenol oxidase and peroxidase activities during ripening of the grape in the Muscate, Rouge, Dimiat Rissing and Alegate varieties. *Ann. Technol. Agr. (Paris)* 17:333.
81. James, W. O. and D. C. Elliott. 1955. Cyanide-resistant mitochondria from the spadix of an Arum. *Nature* 175:89.
82. Jones, R. L. 1973. Gibberellins: Their physiological role. *Ann. Rev. Plant Physiol.* 24:571-598.
83. Kader, A. A., L. L. Morris, and J. M. Lyons. 1973. Effect of abscisic acid on ripening of tomatoes fruits. *HortScience* 8(3):28.
84. Kay, E., L. M. Shannon, and J. Y. Lew. 1967. Peroxidase isoenzymes from horseradish roots II. Catalytic properties. *J. Biol. Chem.* 242:2470-2473.
85. Kende, H. and G. Gardner. 1976. Hormone binding in Plants. *Ann. Rev. Plant Physiol.* 27:267-290.
86. Khudairi, A. K. 1972. The ripening of tomatoes. *American Scientist* 60:696-707.
87. Khudairi, A. K. and O. P. Arboleda. 1971. Phytochrome-mediated carotenoid biosynthesis and its influence by plant hormones. *Physiol. Plantum.* 24:18-22.
88. Kidd, F. and C. West. 1935. The course of respiratory activity throughout the life of an apple. In: Report of the food investigation board for the year 1924. *Gt. Brit. Dept. Sci. Indust. Res. pp.* 27-34.
89. Kitagawa, H., A. Sugiura, and M. Sugiyama. 1966. Effects of GA spray on storage quality of kaki. *HortScience* 1(2):59.
90. Klämbt, D. 1976. Cytokinin effects on protein synthesis of in vitro systems of higher plants. *Plant and Cell Physiol.* 17:73-76.
91. Klepper, L. and R. H. Hageman. 1969. The occurrence of nitrate reductase in apple leaves. *Plant Physiol.* 44:110-114.
92. Knypl, J. S. and K. M. Chylinska. 1974. Removal of IAA-oxidase inhibitors from carrot root extracts by grinding with insoluble polyvinylpyrrolidone. *Biochem. Physiol. Pflanzen (BPP)* 166(4):333-343.
93. Ku, H. S., S. F. Yang and H. K. Pratt. 1969. Ethylene formation from α -keto- γ -methylthiobutyrate by tomato fruit extracts. *Phytochemistry* 8:567-573.

94. Ku, H. S., S. F. Yang, and H. K. Pratt. 1970. Inactivity of apoperoxidase in indoleacetic acid oxidation and in ethylene formation. *Plant Physiol.* 45:358-359.
95. Ku, H. S., S. F. Yang, and H. K. Pratt. 1970. Ethylene production and peroxidase activity during tomato fruit ripening. *Plant and Cell Physiol.* 11:241-246.
96. Kuhive, A. A. 1969. Enzymatic activity of apple leaves and fruit. *Dokl. Akad. Nauk Azerb SSR* 25:95 (in: Chemical abstract 71:88405).
97. Lau, O. and S. F. Yang. 1974. Synergistic effect of calcium and kinetin on ethylene production by mung bean hypocotyl. *Plants* 118:1-6.
98. Lau, O. and S. F. Yang. 1975. Interaction of kinetin and calcium in relation to their effect on stimulation of ethylene production. *Plant Physiol.* 5:738-740.
99. Lau, O. and S. F. Yang. 1976. Stimulation of ethylene production in mung bean hypocotyls by cupric ion, calcium ion, and kinetin. *Plant Physiol.* 57:88-92.
100. Lee, T. T. 1974. Cytokinin control in subcellular localization of indoleacetic acid oxidase and peroxidase. *Phytochemistry* 13:2445-2455.
101. Leshem, Y. 1971. Absciscic acid as a ribonuclease promotor. *Physiol. Plantum* 24:85-89.
102. Leshem, Y. and L. Schwaz. 1972. The selective effect of absciscic acid on ribonucleic acid components. *Physiol. Plantum* 26:328-331.
103. Lipton, W. J. and M. J. Ceponis. 1962. Retardation of senescence and stimulation of oxygen consumption in head lettuce treated with N⁶-benzyladenine. *Proc. Am. Soc. Hort. Sci.* 81:379-384.
104. Loomis, W. D. and J. Battaile. 1966. Plant phenolic compounds and the isolation of plant enzymes. *Phytochemistry* 5:423-438.
105. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193:265-275.
106. Maclean, D. C. and R. R. Dedolph. 1962. Effect of N⁶-Benzylaminopurine on postharvest respiration of Chrysanthemum morifolium and Dianthus caryophyllus. *Bot. Gaz.* 124:20-21.
107. Macnicol, P. K. 1966. Peroxidase of the Alaska pea (Pisum sativum L.): Enzymic properties and distribution within the plant. *Arch. Biochem. Biophys.* 117:347-456.

108. Mapson, L. W. 1970. Biosynthesis of ethylene and the ripening of fruit. *Endeavour* 29:29-34.
109. Matto, A. K. and V. V. Modi. 1969. Ethylene and ripening of mangoes. *Plant Physiol.* 44:308-310.
110. Maxie, E. C. and J. C. Crane. 1967. 2,4,5-Trichlorophenoxyacetic acid: Effect on ethylene production by fruits and leaves of fig tree. *Science* 155:1548.
111. Mayak, S. and D. R. Dilley. 1976. Regulation of senescence in carnation (*Dianthus caryophyllus*): Effect of abscisic acid and carbon dioxide on ethylene production. *Plant Physiol.* 58:663-665.
112. McCune, D. C. 1962. Multiple peroxidases in corn. *Ann. N.Y. Acad. Sci.* 94:723-730.
113. McCune, D. C. and A. W. Galston. 1959. Inverse effect of gibberellin on peroxidase activity and growth in dwarf strains of peas and corn. *Plant Physiol.* 34:416-418.
114. McGlasson, W. B., H. C. Dostal, and E. C. Tigchelaar. 1975. Comparison of proylene-induced responses of immature fruit of normal and 'rin' mutant tomato. *Plant Physiol.* 55:218-222.
115. McGlasson, W. B., J. K. Palmer, M. Vendrell, and C. J. Brady. 1971. Metabolic studies with banana fruit slices II. Effects of inhibitors on respiration, ethylene production, and ripening. *Aust. J. Biol. Sci.* 24:1103-1114.
116. McGlasson, W. B., B. W. Poovaiah, and H. C. Dostal. 1975. Ethylene production and respiration in aging leaf segments and in disk of fruit tissues of normal and mutant tomatoes. *Plant Physiol.* 56:547-549.
117. Milborrow, B. V. 1969. The occurrence and function of ABA in plants. *Sci. Prog. Oxf.* 57:533-558.
118. Miller, C. O. 1962. Kinetin and related compounds in plant growth. *Ann. Rev. Plant Physiol.* 21:359-384.
119. Mitchell, J. W. and P. C. Marth. 1950. Growth regulating substances in horticulture. *Ann. Rev. Plant Physiol.* 1:125-140.
120. Mizrahi, Y., H. C. Dostal, W. B. McGlasson, and J. H. Cherry. 1975. Effect of abscisic acid and benzyladenine on fruits of normal and 'rin' mutant tomatoes. *Plant Physiol.* 56:544-546.
121. Mizrahi, Y., H. C. Dostal, W. B. McGlasson, and J. H. Cherry. 1975. Transplantation studies of immature fruit normal and 'rin' and 'nor' mutant tomatoes. *Plant Physiol.* 55:218-222.

122. Nougarede, A. 1971. Observations nouvelles sur les lieux de peroxydation de la 3,3'-diaminobenidize (DAB) dans les cals obtenu a partir de la souche saurage de l'Acer pseudoplatanus. L. C. R. Acad. Sci. series D, Paris 273:864-867.
123. Panova, R. and L. Bozova. 1975. Variation in the activity of some oxidizing ferment in growing and ripening apple and pear fruits. Gradinar Lozar Nauk 125:28-35. (in: Biological Abstract 61:Entry 33653).
124. Pantastico, ER. B., N. D. Bondad and D. B. Mendoza. 1972. Regulation of fruit ripening II. Chemical treatments. Philippine Agric. 55:375.
125. Plesnicar, M., W. D. Bonner, Jr., and B. T. Storey, 1967. Peroxidase associated with higher plant mitochondria. Plant Physiol. 42:366-370.
126. Poovaiah, B. W., U. Mizrahi, H. C. Dostal, J. H. Cherry, and A. C. Leopold. 1975. Water permeability during tomato fruit development in normal and 'rin' non-ripening mutant. Plant Physiol. 56:813-815.
127. Pratt, H. K. and J. D. Goeschl. 1969. Physiological roles of ethylene in plants. Ann. Rev. Plant Physiol. 20:541-584.
128. Ranadive, A. S. and N. F. Haard. 1971. Differential extraction of peroxidase from ripening pear fruit. Presented at the 31st Annual Meeting of Institute of Food Technologists in New York (paper No. 89).
129. Reid, M. S. and H. K. Pratt. 1970. Ethylene and the respiration climacteric. Nature 226:976-977.
130. Reid, M. S. and H. K. Pratt. 1972. Effect of ethylene on potato tuber respiration. Plant Physiol. 49:252-255.
131. Richmond, A. E. and J. B. Biale. 1966. Protein synthesis in avocado fruit tissue. Arch. Biochem. Biophys. 115:211-215.
132. Richmond, A. E. and A. Lang. 1957. Effect of kinetin on protein content and survival of detached Xanthium leaves. Science 125:650.
133. Robinson, R. W. and M. L. Tomes. 1968. Ripening inhibitor: A gene with multiple effect on ripening. Tomato Genet. Co-op. Rept. 18:36-37.
134. Russo, L., H. C. Dostal, and A. C. Leopold. 1968. Chemical regulation of fruit ripening. BioScience 18:109.
135. Sacher, J. A. 1966. Permeability characteristics and amino acid incorporation during senescence ripening of banana tissue. Plant Physiol. 41:701-708.

136. Sacher, J. A. 1973. Senescence and postharvest physiology. *Ann. Rev. Plant Physiol.* 24:197-224.
137. Saunders, P. F. and R. H. Poulson. 1968. Biochemical studies on the possible mode of action of abscisic acid: An apparent allosteric inhibition of invertase activity. *in: Biochemistry and Physiology of Plant Growth Substance*, pp. 1581-1591. (P. Wightman and E. Setterfield, ed.), Runge Press Ottawa.
138. Schneider, E. A. and F. Wightman. 1974. Metabolism of auxin in higher plants. *Ann. Rev. Plant Physiol.* 25:487-513.
139. Scott, P. C. and A. C. Leopold. 1967. Opposing effect of gibberellin and ethylene. *Plant Physiol.* 42:1021-1022.
140. Scrubb, L. A., A. K. Chakravorty, and M. Shaw. 1972. Changes in the ribonuclease activity of flax cotyledons following inoculation with flax rust. *Plant Physiol.* 50:73-79.
141. Seevers, P. M., J. M. Daly, and F. F. Catedral. 1971. The role of peroxidase isozymes in resistance to wheat stem rust disease. *Plant Physiol.* 48:353-360.
142. Sequeira, L., and L. Mineo. 1966. Partial purification and kinetics of indoleacetic acid oxidase from tobacco roots. *Plant Physiol.* 41:1200-1208.
143. Shannon, L. M. 1968. Plant isoenzyme. *Ann. Rev. of Plant Physiol.* 19:187-210.
144. Shannon, L. M., E. Kay and J. Y. Lew. 1966. Peroxidase isoenzyme from horseradish roots I. Isolation and physical properties. *J. Biol. Chem.* 241:2166-2172.
145. Shaw, M. and M. S. Manocha. 1965. Structure in detached senescing wheat leaves. *Can. J. Botany* 43:747-755.
146. Shinshi, H. and M. Noguchi. 1975. Relationships between peroxidase IAA oxidase and polyphenol oxidase. *Phytochemistry* 14:1255-1258.
147. Siegel, B. Z. and A. W. Galston. 1967. Indoleacetic acid oxidase activity as peroxidase. *Science* 157:1557-1559.
148. Skoog, F. and D. J. Armstrong. 1970. Cytokinins. *Ann. Rev. Plant Physiol.* 21:359-384.
149. Solomos, T. and G. G. Laties. 1974. Similarities between the actions of ethylene and cyanide in initiating the climacteric and ripening of avocados. *Plant Physiol.* 54:506-511.

150. Solomos, T. and G. G. Laties. 1975. The mechanism of ethylene and cyanide action in triggering the rise in respiration in potato tubers. *Plant Physiol.* 55:73-78.
151. Solomos, T. and G. G. Laties. 1976. Effects of cyanide and ethylene on the respiration of cyanide-sensitive and cyanide resistant plant tissues. *Plant Physiol.* 58:47-50.
152. Spaeth, S. C. and N. C. Maravolo. 1973. Partial purification and characterization of an auxin-degrading enzyme in the liverwort Marchantia polymorpha L. *Bot. Gaz.* 134(4): 274-282.
153. Spencer, M. S. 1965. Fruit ripening. in: *Plant Biochemistry* (J. Bonner and J. E. Varner, eds.). Academic Press, New York, pp. 793-825.
154. Srivastava, B. I. S. 1967. Cytokinins in plants. *Int. Rev. of Cytology* 22:350-374.
155. Srivastava, O. P. and R. B. Van Huystee. 1973. Evidence for close association of peroxidase, polyphenol oxidase, and IAA oxidase isoenzyme of peanut suspension culture medium. *Can. J. Botany* 51:2207-2215.
156. Stahman, M. A., B. C. Clare, and W. Woodbury. 1966. Increased disease resistance and enzyme activity induced by ethylene and ethylene production by Black Rot infected sweet potato tissue. *Plant Physiol.* 41:1505.
157. Stenvers, N. and J. Bruinsma. 1975. Ripening of tomato fruits at reduced atmospheric and partial oxygen pressures. *Nature* 253:532-533.
158. Storey, B. T. 1976. Respiratory chain of plant mitochondria XVIII. Point of interaction of the alternate oxidase with the respiratory chain. *Plant Physiol.* 58:521-525.
159. Teatolia, S. S., R. S. Tripathi, and R. N. Singh. 1972. Effect of growth substances on ripening and quality of guava (Psidium quajava L.). *J. Food Sci. Technol.* 9:38-39.
160. Tingwa, P. O. and R. R. Young. 1975. The effect of indole-3-acetic acid and other growth regulators on ripening of avocado fruits. *Plant Physiol.* 55:937-940.
161. Tuli, V., R. R. Dedolph and S. H. Wittwer. 1962. Effect of N⁶-benzyladenine and dehydroacetic acid on the storage behavior of cherries and strawberries. *Mich. Agric. Exp. Stn. Q. Bull.* 45(2):223-226.

162. Ulrich, R. 1958. Postphysiology of fruit. *Ann. Rev. Plant Physiol.* 9:385-416.
163. Urath, C. R. 1974. The commercial implications of GA₄, 7 plus BA for improving shape and yield of Delicious apple. *J. Am. Soc. Hort. Sci.* 99(4):381-384.
164. Van Herk, A. W. H. 1937. Chemical process in *Sauromatum* flowers. *Proc. Acad. Sci. Amsterdam* 40:709-719.
165. Van Overbeek, J., J. E. Loeffler and M. I. Mason. 1967. Dormin (Abscissin II) inhibitor of plant DNA synthesis. *Science* 156:1497-1499.
166. Varner, J. E. 1961. Biochemistry of senescence. *Ann. Rev. Plant Physiol.* 12:245-264.
167. Vendrell, M. 1969. Reversion of senescence: Effects of 2,4-D and IAA on respiration, ethylene production and ripening in banana slices. *Aust. J. Biol. Sci.* 23:601-610.
168. Vendrell, M. 1970. Relationship between internal distribution of oxogenous auxins and accelerated ripening of banana fruit. *Aust. J. Biol. Sci.* 23:1133-1142.
169. Vendrell, M. 1970. Acceleration and delay of ripening in banana fruit tissue by gibberellic acid. *Aust. J. Biol. Sci.* 23:553-559.
170. Wade, N. L. and C. J. Brady. 1971. Effects of kinetin on respiration, ethylene production and ripening of banana fruit slices. *Aust. J. Biol. Sci.* 24:165-167.
171. Wang, C. Y. and E. Hansen. 1970. Differential response to ethylene in respiration and ripening of immature 'Anjou' pears. *J. Am. Soc. Hort. Sci.* 95:314-316.
172. Wang, C. Y. and W. M. Mellenthin. 1972. Maturation of 'Anjou' pears in relation to chemical composition and reaction to ethylene. *J. Am. Soc. Hort. Sci.* 97:9-12.
173. Windus, N. D., V. G. Shutak and R. E. Cough. 1976. Carbon dioxide and ethylene evolution by highbush blueberry fruit. *HortScience* 11(5):515.
174. Workman, M., H. K. Pratt and L. L. Morris. 1957. Studies on the physiology of tomato fruit. I. Respiration and ripening behavior at 20 degree C as related to date of harvest. *Proc. Am. Soc. Hort. Sci.* 69:352-365.

175. Yang, S. F. 1967. Biosynthesis of ethylene: Ethylene formation from methional by horseradish peroxidase. Arch. Biochem. Biophys. 122:481-487.
176. Zobel, R. W. 1973. Some physiological characteristics of the ethylene requiring tomato mutant diageotropica. Plant Physiol. 52:385-389.

BIOGRAPHICAL SKETCH

The author, Shue-cheng Lin, was born on May 28, 1944, in Taiwan, Republic of China. He received his secondary education at Chu-shan middle school and Viator catholic high school in Taichung during the years of 1956-1962. He attended the college of agriculture, Chung-hsing University, from 1962-1966 and was offered the degree of Bachelor of Science in agriculture in June, 1966. Immediately after graduation, he joined the Chinese Army as an R.O.T.C. officer for one year and was discharged as a second lieutenant.

In December, 1967, he was assigned as an assistant specialist in Taitung Agricultural Improvement Station when he passed the Civil Service Board Examination.

He started his graduate work in August, 1968, at the National Taiwan University and received the degree of Master of Science in agriculture in August, 1970.

Then, he worked at Taiwan Agricultural Research Institute as an assistant specialist. He was promoted to an associate specialist three years later and to a senior specialist when he passed the High Rank Civil Service Board Examination in 1974.

He entered the University of Florida in September, 1974, and completed his work towards the Degree of Doctor of Philosophy in June, 1977. He was sponsored with a fellowship from the government of the Republic of China during that period.

He is a member of Alpha Zeta, the American Society of Plant Physiologists, American Society for Horticultural Science, and Chinese Society for Horticultural Science.

He is married to the former Lih-shoue Shih of Yuanlin, Taiwan, and has two sons, Shou-wei Lin and Shou-zen Lin.

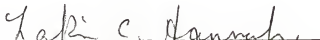
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Dwain D. Gull, Chairman
Associate Professor of Vegetable Crops

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Buford D. Thomson
Professor of Vegetable Crops

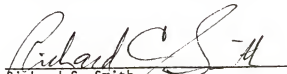
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Larkin C. Hannah
Assistant Professor of Vegetable Crops

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

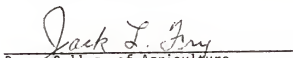

Harold B. Clark
Professor of Food and Resource
Economics

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Richard C. Smith
Professor of Botany

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1978


Dean, College of Agriculture

Dean, Graduate School